The effect of hyperphenylalaninaemia on glycine metabolism in developing rat brain

Charles E. ISAACS* and Olga GREENGARD

Departments of Paediatrics and Pharmacology, Mount Sinai School of Medicine of the City University of New York, New York, NY 10029, U.S.A.

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The brains of 3–16-day-old rats that were rendered hyperphenylalaninaemic by daily injections of α -methylphenylalanine plus phenylalanine were subjected to biochemical analysis. Fluctuations throughout the treatment period in the concentrations of branched-chain amino acids, methionine and serotonin were in agreement with the known interference of excess plasma phenylalanine with transport. The glycine content, however, became abnormal only by day 5, remained so through the treatment, and the elevation was equally apparent at 4, 8 or 24 h after the last daily injections. On the last day of treatment there were small increases in the taurine, glutamate, aspartate and 4-aminobutyrate concentrations, attributable mainly to the diencephalon or brain stem. After day 3 of treatment there were persistent elevations in the specific activity of phosphoserine phosphatase and glycine synthase (but not serine hydroxymethyltransferase) of the brain in each of the regions analysed. The observations indicate that chronic hyperphenylalaninaemia interferes with the normal regulation of intracerebral glycine metabolism during a critical period of early postnatal development, and suggest that the resulting excess in this amino acid (particularly marked in the cortex) contributes to the behavioural abnormalities that these animals exhibit in later life.

α -Methylphenylalanine, an effective suppressor of hepatic phenylalanine hydroxylase (EC 1.14.16.1) (Greengard *et al.*, 1976), has been utilized in some investigations to imitate in rats the metabolic defect associated with the inborn human disease of phenylketonuria. α -Methylphenylalanine is devoid of some of the side effects of the previously used suppressor, *p*-chlorophenylalanine. Infant rats rendered hyperphenylalaninaemic with daily injections of α -methylphenylalanine plus phenylalanine grow at a normal rate, and exhibit lasting behavioural abnormalities in later life (DelValle *et al.*, 1978; Lane *et al.*, 1979). Particularly relevant to biochemical studies on the central nervous system is that α -methylphenylalanine, in contrast with *p*-chlorophenylalanine, does not cause brain polyribosomes to disaggregate, nor is it toxic to neuroblastoma cells in culture (Kelly & Johnson, 1978).

Our first study of the impact of sustained hyperphenylalaninaemia on the chemical composition of immature rat brain showed that this condition does not interfere with the ontogeny of enzymes in glycolysis (hexokinase, pyruvate kinase),

respiration (succinate dehydrogenase) or glutamate metabolism (glutamate decarboxylase and glutamate aminotransferase). Developmental changes in β -hydroxybutyrate dehydrogenase and malate-NADP⁺ dehydrogenase were also normal (DelValle *et al.*, 1978). The only deviation noted in that study was an elevation in the specific activity of cerebral phosphoserine phosphatase. The possible significance of this finding was reinforced when, in the course of detailed studies on the cerebral free amino acid profile (Dienel, 1977), elevations in glycine content were noted in the brains of rat models for phenylketonuria (Dienel & Greengard, 1977). Exploration of this phenomenon was further motivated by the fact that glycine accumulation in the brain is thought to be responsible for the neurological and mental defects associated with another inborn disease, non-ketotic hyperglycinaemia (Perry *et al.*, 1975). The results, in harmony with preliminary studies (Greengard *et al.*, 1979), suggest that hyperphenylalaninaemia, independently from its known effects on the transport of some amino acids, interferes with the intercerebral regulation of glycine metabolism during early postnatal life.

* To whom reprint requests should be addressed.

Experimental

α -Methylphenylalanine, ($\pm$)-L-tetrahydrofolic acid and *O*-phospho-D-serine were obtained from Sigma (St. Louis, MO, U.S.A.), sucrose (special enzyme grade) was purchased from Schwarz/Mann (Orangeburg, NY, U.S.A.), [$1\text{-}^{14}\text{C}$]glycine (sp. radioactivity 56.7 Ci/mol) and L-[$3\text{-}^{14}\text{C}$]serine (sp. radioactivity 56 Ci/mol) from Amersham (Springfield, IL, U.S.A.).

Rats were of the Albino Fischer (CDF) strain. From postnatal day 3 to postnatal day 16 they received, as described by DelValle *et al.* (1978), one daily subcutaneous injection containing α -methylphenylalanine (24 $\mu\text{mol}/10\text{g}$ body wt.) and phenylalanine (52 $\mu\text{mol}/10\text{g}$ body wt.). Control rats were injected with the same volume (0.2 ml/10 g body wt.) of 0.9% NaCl. Biochemical studies were done on freshly obtained tissues. Brains were kept at 0–4°C during the 4–5 min required for dissection. Protein and serotonin concentrations were determined by the methods of Lowry *et al.* (1951) and Curzon & Green (1970) respectively. Amino-acid concentrations were measured with a Beckman 120C automatic analyser.

Enzyme assays

Tissues were homogenized in 9 vol. of 0.32 M-sucrose or in 3 vol. of 0.15 M-KCl. Where indicated, Triton X-100 (final concentration 0.5%) was added and the homogenate was allowed to stand at 4°C for 30 min. Glycine synthase (EC 2.1.2.10) was assayed by the method of Bruin *et al.* (1973). Sucrose homogenates of the brain or parts thereof were layered on 0.5 M-sucrose and centrifuged at 270 g for 10 min. The supernatant, centrifuged at 25 000 g for 30 min, yielded the mitochondrial pellet, which was suspended in 0.32 M-sucrose (0.5 ml/g of brain).

The assay of serine hydroxymethyltransferase (EC 2.1.2.1) in sucrose homogenates containing Triton was based on the method of Taylor & Weissbach (1965). The assay mixture contained 0.06 M-potassium phosphate buffer, pH 7.4, 0.16 mM-pyridoxal 5'-phosphate, 0.04 M- β -mercaptoethanol, 0.01 M-[$3\text{-}^{14}\text{C}$]serine and 0.26 mM-($\pm$)-L-tetrahydrofolic acid. The phosphoserine phosphatase (EC 3.1.3.3) activity of supernatant fluids, obtained by the centrifugation of homogenates (containing sucrose and Triton) at 105 000 g for 30 min, was assayed by the method of Knox *et al.* (1969). For assay of the soluble serine hydratase (EC 4.2.1.13) activity (Greengard & Dewey, 1967), tissues were homogenized in 0.15 M-KCl and centrifuged at 105 000 g for 30 min.

Results

In response to the daily injection of α -methylphenylalanine plus phenylalanine to 3–16-day-old

rats, the plasma concentrations of phenylalanine are 20–30-fold above normal for 18 h every day, and decline to normal just before the next injection (Greengard *et al.*, 1976; DelValle *et al.*, 1978). Changes in brain constituents that occur over several days, as well as those that may undergo daily fluctuations, were of interest in the present study. The analyses were thus carried out at various hours on one treatment day (day 5), as well as in the eighth hour of various days in the course of treatment.

Fig. 1 compares changes that occur on day 5 of treatment in the cerebral concentrations of selected amino acids. Phenylalanine attained a maximal value by 4 h; this was accompanied by a decrease, followed by a recovery in the concentration of methionine and valine (and also leucine and isoleucine; results not shown). Glycine, however, remained at a steady, elevated concentration throughout the day.

Fig. 1. Daily fluctuations in the concentration of some amino acids in the brain of hyperphenylalaninaemic rats. Symbols: ○ and ●, phenylalanine; □ and ■, glycine; ▽ and ▼, tyrosine; △ and ▲, valine; ◇ and ◆, methionine. Open symbols refer to rats injected once a day (from postnatal day 3 to postnatal day 8) with α -methylphenylalanine plus phenylalanine and killed 4, 8, 20 and 24 h after the last injection; the 8 h points are means (see Table 2), the others refer to individual animals. Control values (filled symbols) are shown for one time point (24 h) only. For measure of variability see standard deviations in Table 2.

The administration of α -methylphenylalanine caused only a 75% decrease in the hepatic phenylalanine hydroxylase activity (Greengard *et al.*, 1976). Therefore, some of the phenylalanine, injected in large amounts, is metabolized, and results in 2–3-fold increases in plasma tyrosine content (Del-Valle *et al.*, 1978). The cerebral tyrosine concentration, however, was near normal for most of each experimental day and underwent a transient rise (with the decline in plasma) a few hours before the next daily injection (Fig. 1).

The injection of α -methylphenylalanine alone has no effect on the serotonin content of brain (Greengard *et al.*, 1976), indicating that in contrast with *p*-chlorophenylalanine (Koe & Weissman, 1966) it does not inhibit the tryptophan hydroxylase activity. The other known factor, interference by excess phenylalanine with the uptake of tryptophan (Pardridge, 1977), must be accountable for the 30% decrease in brain serotonin at 4 h (and a recovery by 24 h) after the last daily injection of α -methylphenylalanine plus phenylalanine (Table 1). The concentration of the breakdown product, 5-hydroxyindoleacetic acid, showed the expected fluctuations. The decrease in serotonin was apparent in each brain region.

Amino acids other than those discussed above show no marked fluctuation in response to the daily injection of phenylalanine plus α -methylphenylalanine in brain or plasma. The rise in phenylalanine concentration, 50-fold at 8 h, is similar to that seen at this time in brain (40 times higher than normal).

The phenylalanine content of the brain did not change significantly with the duration of treatment.

Fig. 2. Changes with age in the glycine and serine content of the brain of normal and hyperphenylalaninaemic rats. The glycine ($\square$ and $\blacksquare$) and serine ($\triangle$ and $\blacktriangle$) content of the brain of control and experimental animals is indicated by filled and open symbols respectively. For additional details see the legend to Table 2.

Table 1. Effect of hyperphenylalaninaemia on brain serotonin concentrations

Analyses of rat brains were done 4 h after the fourth and ninth daily injections of α -methylphenylalanine plus phenylalanine or NaCl and 4 h as well as 24 h after the sixth daily injection of these substances. Values are means $\pm$ S.D. for results on three different brains, except where indicated. Abbreviations used: 5HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid.

Age (days)	Time after injection (h)			Content (nmol/g)	
				Control	Experimental
6	4	5HT	Whole brain	1.13 $\pm$ 0.05	0.80 $\pm$ 0
		5-HIAA		0.35 $\pm$ 0.03	0.11 $\pm$ 0.03
8	4	5HT	Whole brain	1.60 $\pm$ 0.09	1.13 $\pm$ 0.14
		5-HIAA		0.95 $\pm$ 0.22	0.43 $\pm$ 0.05
8	24	5HT	Whole brain	1.50 $\pm$ 0.05	1.50 $\pm$ 0.15
		5-HIAA		1.25 $\pm$ 0.08	0.63 $\pm$ 0.10
11	4	5HT	Whole brain	1.62 $\pm$ 0.11	1.13 $\pm$ 0.23
		5-HIAA		1.11 $\pm$ 0.03	0.47 $\pm$ 0.20
		5HT	Brain stem	3.67 $\pm$ 0.15	2.13 $\pm$ 0.19
		5-HIAA		3.46 $\pm$ 0.56	0.85 $\pm$ 0.13
		5HT	Cerebellum	1.61 $\pm$ 0.18	1.37 $\pm$ 0.20
		5-HIAA		1.19 $\pm$ 0.10	0.41 $\pm$ 0.15
		5HT	Cortex	1.23 $\pm$ 0.22	0.80 $\pm$ 0.12
		5-HIAA		0.61 $\pm$ 0.09	0.22 $\pm$ 0.07
		5HT	Diencephalon	1.81 $\pm$ 0.25	1.27, 1.03
		5-HIAA		1.55 $\pm$ 0.61	0.62, 0.54

Table 2. *Changes in the free-amino-acid profile of the immature rat brain during chronic hyperphenylalaninaemia*
Analyses were done 8 h after the last injection of α -methylphenylalanine plus phenylalanine (E) or NaCl (C). The 16-day values refer to individual brains; the others are means ($\pm$ S.D.) of results on three (8-day) or four (12–13-day) brains. Abbreviations used: Tau, taurine; GABA, 4-aminobutyrate; Cysta, cysteic acid.

Age (days) ... Day of treatment ...	Amino acid content (μ mol/g of brain)					
	8		12–13		16	
	5		9–10		13	
	C	E	C	E	C	E
Phe	0.07 $\pm$ 0.01	2.94 $\pm$ 0.59	0.08 $\pm$ 0.01	2.76 $\pm$ 0.50	0.06, 0.08	2.01, 2.25
Gly	1.61 $\pm$ 0.11	2.23 $\pm$ 0.22	1.07 $\pm$ 0.09	2.02 $\pm$ 0.15	0.72, 0.88	1.56, 1.32
Ser	1.26 $\pm$ 0.09	1.43 $\pm$ 0.13	1.20 $\pm$ 0.16	1.40 $\pm$ 0.08	1.21, 0.99	1.30, 1.44
Ala	0.86 $\pm$ 0.09	0.60 $\pm$ 0.10	0.82 $\pm$ 0.70	0.66 $\pm$ 0.05	0.66, 0.52	0.56, 0.72
Val	0.17 $\pm$ 0.09	0.07 $\pm$ 0.01	0.19 $\pm$ 0.05	0.15 $\pm$ 0.01	0.37, 0.25	0.08, 0.08
Leu	0.20 $\pm$ 0.04	0.12 $\pm$ 0.05	0.20 $\pm$ 0.04	0.13 $\pm$ 0.03	0.16, 0.25	0.20, 0.19
Ile	0.08 $\pm$ 0.05	0.03 $\pm$ 0.01	0.06 $\pm$ 0.02	0.03 $\pm$ 0.01	0.09, 0.05	0.02, 0.03
Glu	7.44 $\pm$ 0.47	6.03 $\pm$ 0.81	6.70 $\pm$ 0.53	6.49 $\pm$ 0.35	6.78, 7.80	8.57, 9.75
Gln	4.16 $\pm$ 0.19	3.7 $\pm$ 0.46	4.20 $\pm$ 0.54	4.03 $\pm$ 0.20	3.39, 2.93	3.64, 4.38
Met	0.10 $\pm$ 0.07	0.05 $\pm$ 0.05	0.05 $\pm$ 0.01	0.04 $\pm$ 0.01	0.08, 0.02	0.02, 0.02
Cysta	0.19 $\pm$ 0.01	0.13 $\pm$ 0.05	0.16 $\pm$ 0.05	0.14 $\pm$ 0.02	0.33, 0.25	0.22, 0.21
Tyr	0.26 $\pm$ 0.03	0.34 $\pm$ 0.06	0.20 $\pm$ 0.02	0.35 $\pm$ 0.04	0.16, 0.26	0.15, 0.23
Asp	3.13 $\pm$ 0.33	2.8 $\pm$ 0.61	2.20 $\pm$ 0.25	2.56 $\pm$ 0.19	3.58, 3.02	4.03, 4.71
Thr	0.82 $\pm$ 0.06	0.81 $\pm$ 0.16	0.82 $\pm$ 0.12	0.93 $\pm$ 0.03	0.72, 0.54	0.93, 0.73
Tau	17.32 $\pm$ 3.39	16.77 $\pm$ 1.31	13.24 $\pm$ 1.06	13.91 $\pm$ 1.18	4.65, 5.49	6.25, 7.41
GABA	1.48 $\pm$ 0.35	1.56 $\pm$ 0.17	1.86 $\pm$ 0.06	1.95 $\pm$ 0.13	1.57, 1.45	1.55, 1.77
Arg	0.15	0.09	0.19 $\pm$ 0.07	0.14 $\pm$ 0.03	—	—
His	0.16	0.14	0.31 $\pm$ 0.04	0.20 $\pm$ 0.01	—	—
Lys	0.24	0.22	0.32 $\pm$ 0.04	0.31 $\pm$ 0.05	—	—

The deficits in methionine or branched-chain amino acid content on day 3 (results not shown) were similar to those on subsequent days (Table 2). The alteration in glycine concentration, however, was not yet evident on day 3 of treatment: 2 days later it was significantly above the control value (Fig. 2).

Since the glycine concentration of normal rat brain is known to decrease during early postnatal life (Bayer & McMurray, 1967), assessment of the experimental results against this changing baseline is perhaps more meaningful. Fig. 2 shows that the rapid decrease of the cerebral glycine in the controls between the ages of 6 and 12 days failed to occur in the hyperphenylalaninaemic animals. The control values for serine remained steady during the developmental period studied, and were always lower than the experimental ones (see Fig. 2). Among non-aromatic amino acids only glycine and serine exhibited consistent increases. On the last day of treatment, but not earlier, the concentrations of taurine, glutamic acid and aspartic acid were also slightly above the control values (see Table 2).

The activities of three enzymes on the pathway of cerebral glycine and serine synthesis, and one that is not involved in this process (serine dehydratase) have now been measured under saturating substrate concentrations *in vitro*. Higher phosphoserine phosphatase activities were previously found in the brains

of hyperphenylalaninaemic than in normal 16- and 33-day-old rats (DelValle *et al.*, 1978). The present results show that this difference is not present 4 or 6 h after the first injection of α -methylphenylalanine plus phenylalanine, but becomes noticeable on day 2 of treatment and persists throughout the experimental period (Table 3). The glycine synthase activity of the experimental brains was consistently above that of control brains of the same age, whereas the activities of serine hydroxymethyltransferase were clearly unaltered by hyperphenylalaninaemia (Table 3).

One purpose of the experiments of Table 4 was to compare in immature and adult brain the distribution of different enzymes of glycine metabolism with each other and with the concentration of some amino acids. The other purpose was to look for hyperphenylalaninaemia-induced changes restricted to a particular region that may have been obscured by analysis of whole-brain preparations.

Previous studies on the brains of normal adult rats showed that the glycine synthase was present at much higher activities in the cortex than in other regions (Daly *et al.*, 1976). The results in Table 4 indicate that the glycine synthase content of cortex relative to cerebellum (or brain stem) increased between day 16 of life and adulthood. The serine hydroxymethyltransferase exhibited no significant

Table 3. *Enzyme activities*

The treatment (see also the legend to Fig. 1) began on postnatal day 3. Values refer to single animals or are means $\pm$ S.D. for results on the numbers of animals given in parentheses. The means of all control (C) and experimental (E) values for glycine synthase (4.1 ± 0.45 and $5.0 \pm 0.53 \mu\text{mol/min per g}$ of brain respectively) are significantly different from each other at $P < 0.001$.

		Enzyme activity (nmol/min per g of brain)						
Day of treatment	...	1	2	4	6	10	12	14
Phosphoserine phosphatase	C	168	162	188	248	248	241 ± 12 (11)	223 ± 23 (3)
	E	160	188	227	284	417	311 ± 15 (11)	289 ± 24 (3)
Glycine synthase	C			3.8	4.9	5.2	4.7	3.7, 4.2
	E			4.4	5.9	6.0	5.5	4.6, 5.3
Serine hydroxymethyltransferase	C					94		58 ± 2 (3)
	E					93		63 ± 2 (3)
Serine dehydratase	C				5.8	6.1	5.8	9.4
	E				4.9	4.1	5.4	8.7

Table 4. *Enzymes and amino acids in various brain regions of the normal and hyperphenylalaninaemic rat brain*

Mean results of analyses on two normal adult brains are shown in parentheses. The other values refer to 16-day-old suckling rats, treated from postnatal day 3 with NaCl (C) or α -methylphenylalanine plus phenylalanine (E); the indicated regions from six (C) or seven to ten (E) individual brains were pooled. One set of such pools was used for enzyme measurements only (see the first value for each enzyme) and the other set for both amino acid and enzyme analyses. Abbreviations used: Cysta, cysteic acid; GABA, 4-aminobutyrate; Tau, taurine.

		Enzyme activity (nmol/min per g) or amino-acid content (nmol/g)									
Brain part	...	Cortex and hippocampus		Diencephalon		Cerebellum		Brain stem			
		C	E	C	E	C	E	C	E		
		(5.91)	(1.37)	(1.09)	(1.46)	(1.87)	(1.75)				
Glycine synthase		4.88	5.79	1.60	1.60	2.08	2.32	1.52	1.91		
		5.20	6.00	1.68	1.50	1.65	1.97	1.33	1.63		
Phosphoserine phosphatase	(256)	222	286	144	214	180	296	153	192		
		267	326	173	239	216	330	177	240		
Phe		0.07	2.01	0.08	2.35	0.06	1.95	0.06	2.48		
Gly		0.48	1.10	0.84	1.58	0.59	0.95	2.30	3.17		
Ser		1.16	1.36	1.06	1.54	0.97	1.46	1.00	1.15		
Glu		8.55	9.69	6.28	10.96	5.52	7.34	5.36	6.39		
Gln		3.20	3.35	2.80	5.85	3.23	4.83	3.28	4.21		
Cysta		0.22	0.096	0.22	0.15	0.65	0.80	0.38	0.28		
Asp		3.69	4.48	2.71	5.25	2.57	3.59	2.88	3.56		
Thr		0.75	0.77	0.47	1.23	0.43	0.91	0.49	0.58		
Tau		6.18	5.70	4.06	14.14	3.73	7.71	2.88	3.07		
GABA		1.44	1.53	2.38	2.27	0.84	1.08	1.47	2.02		

change with age; as in adult rats (Daly & Aprison, 1974), it was somewhat more concentrated in the cerebellum and brain stem than in cortex and diencephalon. The distribution of phosphoserine phosphatase was unlike that of either glycine synthase or serine hydroxymethyltransferase, although the cortex contained somewhat more than did other regions (Table 4).

Small elevations in glycine synthase activity (seen also in whole-brain preparation; Table 3) were equally apparent in cortex, cerebellum and brain stem, but absent in diencephalon (Table 4). The

phosphoserine phosphatase activity of all four areas increased appreciably, perhaps more so in the cerebellum than elsewhere. The protein concentration (mg/g) of brain parts (or whole brain) of experimental animals did not differ from those of the control ones.

Data on some of the 16 amino acids measured in different brain parts of 16-day-old rats are shown in the last part of Table 4. No significant regional differences were seen in the concentration of phenylalanine or serine or in their increase in response to treatment. Valine, leucine, isoleucine and

Table 5. *Increments in concentration of putative neurotransmitters in brain regions of hyperphenylalaninaemic rats*
The values, referring to 16-day-old rats (i.e. to day 14 of treatment), were calculated from data in Table 5. The asterisks, one in each horizontal line, serve to indicate the brain part in which the absolute concentration of that amino acid is highest. Abbreviations used: Tau, taurine; GABA, 4-aminobutyrate.

	Increase (%)				
	Cortex	Diencephalon	Cerebellum	Brain stem	Whole brain
Gly	129	88	61	38*	80
Asp	21*	94	40	24	32
Glu	13*	75	33	19	24
GABA	6	0*	29	37	10
Tau	0*	248	107	10	35

methionine (as well as alanine and tyrosine) were also evenly distributed, and the deficits caused by hyperphenylalaninaemia (see Fig. 1 and Table 2) were similar in each region (results not shown). The increase of glycine in response to hyperphenylalaninaemia was apparent in each region. To facilitate comparison of the distribution of these increments with those exhibited by additional amino acids that showed a positive response to hyperphenylalaninaemia, the percentage changes are listed in Table 5. Only for glycine was the disparity maximal in cortex. For glutamic acid and aspartic acid in cerebellum, and for 4-aminobutyrate in the brain stem, 30–40% increments were seen, whereas taurine exhibited 100–200% increases in the diencephalon and cerebellum. We do not know the day of treatment at which the changes in these structures become manifest; in whole-brain preparations increments of glycine were apparent from day 5 on, whereas those in glutamic acid, 4-aminobutyrate, taurine and aspartic acid were detectable on the last day of treatment only (see Table 2).

Discussion

In infant rats treated by the present method the elevation of the phenylalanine concentration in plasma and brain (Greengard *et al.*, 1976; DelValle *et al.*, 1978; Table 2) was similar to that seen in phenylketonuric children (Knox, 1972). Some direct consequences of such elevations, exemplified here by diminutions of the cerebral concentration of branched-chain amino acids and of the tryptophan metabolite serotonin, have been extensively documented (McKean, 1972; Antonas & Coulson, 1975; McKean *et al.*, 1968; Carver, 1965). In fact, the inhibition by excess phenylalanine of the uptake of amino acids sharing the same transport systems is the only well understood mechanism underlying chemical abnormalities in the brain of the hyperphenylalaninaemic organism. Since there is no obvious relationship between glycine and phenylalanine with respect to either transport or meta-

bolism, studies of abnormalities in the brain or cerebrospinal fluid of phenylketonuric subjects have unfortunately not included glycine. It was the increased phosphoserine phosphatase activity of the brains of hyperphenylalaninaemic rats that directed our attention to this subject. Treatment with phenylalanine plus either *p*-chlorophenylalanine or α -methylphenylalanine evoked this increase (DelValle *et al.*, 1978). The elevation in cerebral glycine, too, was common to both these rat models of phenylketonuria, and was also apparent after several days of treatment with high doses of phenylalanine alone (Dienel & Greengard, 1977). The abnormal regulation of the glycine content of infant rat brain is thus attributable to hyperphenylalaninaemia *per se* rather than to a side effect of α -methylphenylalanine.

Changes in the free-amino-acid profile of brain, which are due to excess phenylalanine competing for a common transport system, could be rapidly reversible and vary with the plasma concentration of phenylalanine. The behaviour of valine, leucine, isoleucine and methionine conform to this pattern (see Fig. 1 and Carver, 1965). A different type of mechanism must underlie the increase in cerebral glycine concentration: it did not exceed the control values until day 5 of treatment, and did not show daily fluctuations. A direct action of phenylalanine (or its metabolites) on the transport of glycine, or on merely the activity of enzymes in its synthesis or degradation, would be expected to evoke a less sluggish and more rapidly reversible response. An action on a time-consuming process, like alteration in the amounts of specific proteins, is more compatible with the observations reported in the present paper. Enzymes are likely candidates, since, at least in normal animals, the glycine content of brain is regulated by intracerebral metabolism rather than by its concentration in plasma (Aprison *et al.*, 1975). The increment in phosphoserine phosphatase indeed preceded that in glycine (Table 3), persisted during the same experimental period and extended to all brain parts whose glycine content exceeded the norm (Table 4).

The inborn human disease of (non-ketotic) hyperglycinaemia is associated with a deficiency in hepatic glycine synthase (Yoshida & Kikuchi, 1969). But since plasma concentrations of glycine have little role in regulating its cerebral concentrations (Aprison *et al.* 1975), elevations in the latter cannot be attributed to the hepatic defect alone. The diminished glycine synthase activity in the brains of these subjects (Perry *et al.*, 1975) may well be the explanation [although alternative possibilities, e.g. a defective membrane transport protein, evidenced in fibroblasts, have been suggested (Revsin & Morrow, 1976)]. However, increased glycine synthase activity, since the reaction is reversible, can also lead to glycine accumulation, and this may contribute to the hyperphenylalaninaemia-induced changes in cerebral glycine content. The role of the increment in phosphoserine phosphatase might be more decisive, since it catalyses an irreversible and probably rate-limiting reaction on the pathway of glycine synthesis.

On day 13 of treatment increases were also noted in glutamic acid, 4-aminobutyrate and aspartic acid, and there was an apparent delay (Table 2) in the normal developmental decrease of taurine (Sturman *et al.*, 1977) as well as in glycine. Common to these putative neurotransmitters is their high concentration in brain (compared with plasma). The possibility that one of the factors involved in regulating their efflux or influx is common, and becomes abnormal during prolonged hyperphenylalaninaemia, can thus not be entirely disregarded.

Glycine is known to be an inhibitory neurotransmitter in the brain stem and spinal cord (Johnston & Iversen, 1971; Aprison & McBride, 1973); evidence for a function in the cortex is restricted to the frog (Davidoff & Adair, 1976). Nevertheless, the severe neurological problems and inevitable mental deterioration of children with non-ketotic hyperglycinaemia are attributed to the 2- to 3-fold elevations in their cerebral glycine concentration (Baumgartner *et al.*, 1969; Perry *et al.*, 1975; Gitzelmann *et al.*, 1977; deGroot *et al.*, 1978). The neurotoxicity of the same degree of elevation (induced in normal animals by the administration of serine plus glycine) was also demonstrated in infant rats, 0–8-day-old animals showing the greatest response (deGroot *et al.*, 1978, 1979). The significant but somewhat smaller (50–100%) increment of glycine in hyperphenylalaninaemic rats caused no obvious neurotoxicity. It prevailed, however, during a period that is 'critical' in the ontogeny of a variety of cerebral functions in the rat, and that includes the week of maximal sensitivity of the central nervous system to glycine specifically. The abnormality in cerebral glycine content induced by early hyperphenylalaninaemia may thus contribute significantly to the deviant behaviour and

learning deficits that the same animals exhibit in later life (DelValle *et al.*, 1978; Lane *et al.*, 1979).

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