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CEREBRAL SEROTONIN REGULATION BY PHENYLALANINE ANALOGUES AND DURING HYPERPHENYLALANINEMIA

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Abstract—Severe hyperphenylalaninemia induced in infant rats by 3 days of treatment with *p*-chlorophenylalanine (*p*-clphe) plus phenylalanine (phe) did not lower the tryptophan concentration of the brain, and the cerebral serotonin (5-HT) deficiency was attributable entirely to the known suppression of tryptophan hydroxylase (TPH) by *p*-clphe. The decrease in 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) was thus no more pronounced than in rats which, treated with *p*-clphe alone, were devoid of hyperphenylalaninemia. Suppression of TPH was found to also underlie the decrease in cerebral 5-HT caused by treatment with α-methylphenylalanine (α-mephe) alone: a 22% loss of midbrain TPH activity was detectable 24 hr after an injection only, reverted toward the normal during the next 2 days, and was clearly unrelated to the weak competitive inhibition of the enzyme by α-mephe *in vitro*. However, α-mephe (unlike *p*-clphe), when administered together with phe, did not suppress TPH, nor did it counterbalance the reduction of cerebral tryptophan uptake by excess phe. Thus the 5-HT diminution in the rat model of phenylketonuria produced by treatment with α-mephe plus phe was attributable to hyperphenylalaninemia and the inhibition of tryptophan transport to the brain. Injection of tryptophan was found to restore the cerebral 5-HT level in the face of persistently severe hyperphenylalaninemia.

McKean's study [1] in 1972 on the autopsied brains of phenylketonuric (PKU) children was the first to provide conclusive evidence for the association of cerebral serotonin (5-HT) and catecholamine depletion with a genetic disease. The primary lesion (phenylalanine hydroxylase deficiency) is restricted to the liver, and the cerebral changes, of which mental retardation is the most devastating one, are secondary to the accumulation of phenylalanine (phe) in the blood [2]. Subsequently discovered variants of the disease, where the metabolic block is in the production of the tetrahydrobiopterin cofactor essential for the hydroxylation of tryptophan and tyrosine, as well as phe, have also been found to be associated with severe neurological abnormalities [3]. The amelioration of these abnormalities by administration of precursors of 5-HT and of catecholamines [4] has confirmed the postulated pathogenic role of deficiencies in these amines in PKU and has resulted in renewed interest in animal models suitable for study of the underlying mechanisms. α-Methylphenylalanine (α-mephe), a suppressor of the hepatic phenylalanine hydroxylase we identified a few years ago [5], was found to be useful for mimicking the defects in human PKU, for its administration to immature rats together with phe maintains severe hyperphenylalaninemia without obvious toxic side-effects [6] and leads to behavioral abnormalities including diminished maze-learning ability [7–9]. Our recent studies of the mechanism whereby this and alternative treatments interfere with the metabolism of catecholamines [10] has now been extended to that of 5-HT. The relevance of such studies, as indicated by recent evidence for the role of 5-HT and catecholamines as humoral agents in normal brain differentiation [11, 12], is not restricted to these rare inborn diseases. Information gathered in genetically normal animals about synthetic or natural regulators, whereby the cerebral levels of 5-HT, dopamine and norepinephrine can be modulated independently of one another, should also be useful in the design of experimental systems necessary for specifying the separate roles of these amines in normal cerebral development.

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MATERIALS AND METHODS

Materials. 5-HT, 5-HIAA, DL-α-methylphenylalanine, and catalase were purchased from the Sigma Chemical Co., St. Louis, MO. DL-6-Methyl-5,6,7,8-tetrahydrobiopterine ($6MPH_4$), *o*-phthalaldehyde and *p*-chlorophenylalanine (*p*-clphe) were purchased from Calbiochem, La Jolla, CA, the Regis Chemical Co., Morton Grove, IL, and the Aldrich Chemical Co., Milwaukee, WI, respectively.

Animals and treatments. Albino Fischer rats, suckled by mothers given standard chow and water *ad lib*, were used throughout these studies. All injections, 0.1 to 0.3 ml per 10 g body weight, were subcutaneous. *p*-Clphe (with or without phe) was sonicated and heated in 0.9% NaCl solution and injected as a fine suspension. α-Mephe and phe were dissolved by heating. Standard doses of α-mephe (24 μmoles/10 g body wt) or *p*-clphe (9 μmoles/10 g body wt) with or without phe (52 μmoles/10 g body wt) were injected once ("acute experiments") or once a day for at least 3 days ("chronic experiments"). The dosages were based on previous studies

describing the suppression of phenylalanine hydroxylase by phe analogues and the treatment schedules required to maintain severe hyperphenylalaninemia [5, 6]. Control animals (littermates) received vehicle only.

Assay of tryptophan and phe in brain and plasma. Brain tissue was homogenized in 12 vol. of 10% trichloroacetic acid and centrifuged at 20,000 *g* for 10 min. Heparinized trunk blood was centrifuged for 5 min; the plasma was deproteinized by mixing with 10% trichloroacetic acid after a 100-fold dilution and centrifuged at 20,000 *g* for 10 min.

The measurement of tryptophan was based on the fluorimetric method of Denckla and Dewey [13] as revised by Bloxam and Warren [14] and Lehmann [15]. The volume of brain supernatant fraction used in the tryptophan assay was 0.1 and 0.2 ml, and that of plasma supernatant fraction was 0.5 to 1.0 ml; 0.2 ml of 2% formaldehyde was added to the tubes, followed by 0.1 ml of 6 mM $FeCl_2$. The samples were then immediately heated at 100° for 1 hr and fluorescence was read at E_{max} 450 nm with activation at 370 nm. Phe, α-mephe and *p*-clphe (up to concentrations of 0.52, 0.44 and 0.16 mM respectively) did not interfere with the assay.

Phe was measured by the fluorimetric method of McCaman and Robins [16] as described by Faulkner [17]. α-Mephe did not interfere with the assay, but in animals treated with *p*-clphe (which did interfere) phe was measured in a Beckman automatic amino acid analyzer.

Assay of 5-HT and 5-HIAA in midbrain or whole brain. The fluorimetric method of Curzon and Green [18], based on the formation of fluorescent complexes when 3.5 substituted indoles are reacted with *o*-phthalaldehydes, was used. Brain tissues were homogenized in cold acidified butanol and centrifuged at 3000 rpm for 5 min. Then 2.5 ml of the supernatant was added to 5 ml *n*-heptane and 0.4 ml of 0.1% cysteine. The aqueous phase was separated by centrifugation at 3000 rpm for 5 min, and 5-HT was then reacted with 0.01% *o*-phthalaldehyde in 10 N HCl. For the determination of 5-HIAA, 5 ml of the organic phase was mixed with 0.6 ml of 0.5 M sodium phosphate buffer (Ph 7.0), centrifuged at 3000 rpm for 3 min, and fluorescence was determined after reaction of the aqueous phase with 0.1% *o*-phthalaldehyde in 10 N HCl. All preparations were assayed immediately or stored under nitrogen gas at −20° for less than 1 week. Fluorescence, at E_{max} 470 nm with activation at 360 nm, was found to increase linearly with 5-HT in the range of 30 to 600 ng and with 5-HIAA in the range of 60 to 600 ng. Standard solutions of 5-HT and 5-HIAA were made up in 0.1% cysteine and in 0.5 M sodium phosphate buffer (pH 7.0) respectively. Experiments in which these standard solutions were added to brain tissue preparations showed that the extraction procedure recovered 95–100% of 5-HT and 5-HIAA.

Measurement of TPH. Rat midbrain was homogenized in 4 vol. of 50 mM Tris-acetate buffer (pH 7.60) containing 10 mM 2-mercaptoethanol, centrifuged at 30,000 *g* for 30 min, and the supernatant fraction was used for the assay of TPH (EC 1.14.16.4). The method, involving fluorimetric measurement of the 5-hydroxyindoles produced, was based on that of Gal and Patterson [19]. In the 1 ml of incubation mixture final concentrations of substances were as follows: 50 mM Tris-acetate (pH 7.6), 1 mM 2-mercaptoethanol, 0.2 mM pargyline, 0.176 mM L-tryptophan, 0.16 mM DL-6-methyl-5,6,7,8-tetrahydropterin hydrochloride ($6MPH_4$), 10 μg catalase and 0.050 to 0.150 ml midbrain homogenate. Under these conditions, no significant activity occurred during incubation in the absence of $6MPH_4$, i.e. serotonin content was the same as that of incubated complete reaction mixtures. Blanks consisting of simultaneously incubated complete reaction mixtures minus $6MPH_4$ were included in each experiment. Reaction was started by addition of the tissue supernatant fraction. After 30 min of incubation at 37° in a shaking water bath, the tubes were placed in boiling water for 5 min and centrifuged at 3000 rpm for 10 min. Then 0.5 ml of the supernatant fraction was added to 0.1 ml of 1% cysteine, 1.15 ml of 10 N HCl and 0.2 ml of 0.01% *o*-phthalaldehyde in 10 N HCl. After heating the reaction mixture at 100° for 15 min, the samples were cooled and fluorescence was read at activation 360 nm and E_{max} 470 nm. Reaction rate was constant up to 30 min of incubation. Recovery of 5-HT added to cofactor-free incubation mixtures was 100%. Addition of α-mephe and phe, at twenty times greater levels than estimated to be present in experimental brains, did not interfere with the enzyme assay. Enzyme activity is expressed as nmoles product per hour per gram tissue.

RESULTS

Diminished levels of 5-HT and 5-HIAA have been found to be present in the brains of fetal [20] and postnatal [7, 21] rats rendered hyperphenylalaninemic by treatment with α-mephe plus phe. Table 1, representing an extension of those results, shows that the extent of the diminutions was similar in the course of the developmental period studied and was comparable to the 44% 5-HT deficiency found in the occipital cortex of PKU subjects [1]. The effect of hyperphenylalaninemia was not cumulative in that the same 5-HT of 5-HIAA deficits resulted from 3 as from 8 or 10 days of treatment (see group marked by an asterisk). In view of these results, subsequent studies of the chronic effect of excess phe and its analogues were carried out in 8 to 11-day-old rats that had been treated for at least 3 days.

It was shown previously that tryptophan administration to normal animals raises the cerebral 5-HT content [22]. To determine whether the effect of chronic hyperphenylalaninemia on 5-HT could be circumvented by precursor supplementation, rats were given standard daily doses of α-mephe plus phe for 2 days and on day 3 this was combined with tryptophan administration. The consequent 20-fold rise in plasma tryptophan concentration did not alter the severity of hyperphenylalaninemia but, as seen from Table 2, the cerebral 5-HT of these animals was at control rather than subnormal concentration. (The very high 5-HIAA level suggests that a smaller amount of tryptophan than injected here would have been sufficient to restore the cerebral phe content.)

Table 1. Effect of chronic hyperphenylalaninemia on the developing brain's serotonin content

Days of age	5-HT (nmoles/g wet wt brain) Control	Experimental	5-HIAA (nmoles/g wet wt brain) Control	Experimental
1	1.26, 1.10		1.04	
6	1.13 ± 0.05	0.80 ± 0.01	0.35 ± 0.03	0.11 ± 0.03
8	1.60 ± 0.09	1.13 ± 0.14	0.95 ± 0.22	0.43 ± 0.05
8*	1.63 ± 0.27	1.05 ± 0.05		
11	1.62 ± 0.11	1.13 ± 0.23	1.11 ± 0.03	0.47 ± 0.20
14	2.00 ± 0.20	1.12 ± 0.08	0.81 ± 0.17	0.27 ± 0.05
16	1.93 ± 0.17	0.89 ± 0.09	1.62 ± 0.43	0.24 ± 0.08

Measurements were made 4–6 hr after the last daily injection of α-mephe plus phe (experimental) or saline (control) as described under Materials and Methods. All animals were treated from 3 days of age except for the asterisked 8-day-old animals which were treated from day 6. Values are means ± SD for three to five animals or refer to a single animal. The data in lines 2, 3 and 5 are from a previous study [21].

Table 2. Reversal of cerebral 5-HT decrease with tryptophan injections after α-mephe plus phe treatment

	5-HT (nmoles/g)	5-HIAA (nmoles/g)
Control	1.61 ± 0.09 (5)	0.90 ± 0.07 (4)
α-Mephe + phe	0.97 ± 0.13 (3)	0.19 ± 0.06 (3)
α-Mephe + phe +tryptophan	1.47 ± 0.11 (4)	3.45 ± 0.17 (4)

Rats were treated for 3 days with α-mephe plus phe and were killed 6 hr after the last injection at 11 days of age. Tryptophan (a total of 3 mg/10 g) was injected 1, 3, and 5 hr after the α-mephe plus phe injection on day 11. Values are means ± SD with the number of animals given in parentheses.

The treatment with α-mephe plus phe resulted in over 20-fold elevation in plasma and brain phe and a 50% decrease in cerebral tryptophan concentration (cf. lines 1 and 2 in Table 3). Similar elevations in phe and a greater cerebral 5-HT deficiency were seen in rats receiving *p*-clphe (instead of α-mephe) plus phe (cf. lines 3 and 4); however, the tryptophan concentration of the brain was not below (but somewhat above) the control value. The known ability of *p*-clphe to inhibit 5-HT synthesis [22] must be the reason for the cerebral 5-HT deficit here since it was the same as in rats (cf. lines 3 and 5) which, treated with *p*-clphe alone, were devoid of severe hyperphenylalaniemia and exhibited significantly higher than normal cerebral trytophan concentration.

The small decrease in 5-HT caused by α-mephe alone (see line 4 of Table 3) may have been due to the slight increase in the plasma phe levels resulting from the 3 days of treatment. However, if given enough time, even a single injection of α-mephe caused some 5-HT deficit. This result, and the very pronounced decrease in 5-HIAA which further attests to the ability of α-mephe to interfere with 5-HT metabolism, are shown by the broken lines in Fig. 1. It may also be seen that the fall and rise in the concentration of these compounds paralleled in time, and can be explained by, the fall and rise that the injected α-mephe caused in TPH activity. To illustrate that the time course of change in midbrain TPH closely resembles the slowly developing suppression and gradual recovery of the hepatic phenylalanine hydroxylase previously shown to follow an injection of α-mephe [6], Fig. 1 also includes data on the latter enzyme.

As shown in Fig. 2, α-mephe had some affinity to TPH; it was a weak competitive inhibitor of the enzyme, with an apparent K_i of 1.2 mM. This finding does not explain, however, the results in Fig. 1. Assuming that the injected α-mephe, 24 μmoles/10 g, is evenly distributed in the body (which, since no method is available for its measurement in tissue extracts, could not in fact be determined), and that none is eliminated in 24 hr (which is unlikely), its

Table 3. Response of cerebral tryptophan and 5-HT concentrations to suppression of hepatic phenylalanine hydroxylase by different methods

Treatment	Plasma Phe (nmoles/ml)		Brain Phe (nmoles/g)		Brain Try (nmoles/g)		Midbrain 5-HT (nmoles/g)	
Saline	143 ± 53	(5)	70 ± 10	(3)	21.0 ± 3.3	(12)	4.46 ± 0.65	(13)
α-Mephe + phe	3130 ± 323*	(4)	2940 ± 59*	(3)	9.5 ± 1.9*	(11)	2.60 ± 0.49*	(9)
p-Clphe + phe	3430 ± 140*	(3)	2100 ± 14*	(3)	23.5 ± 6.7	(13)	2.04 ± 0.61*	(11)
α-Mephe	240 ± 39	(3)	310 ± 0.16*	(3)	16.5 ± 3.7	(3)	3.93 ± 0.46*	(9)
p-Clphe	197 ± 21	(3)	104, 140		28.4 ± 4.5*	(13)	1.97 ± 0.61*	(10)

The measurements, on 7 to 10-day-old rats, were done 4–6 hr after the last of three injections (one per day) of standard doses (see Materials and Methods) of the indicated substances.
* Significant difference ($P < 0.01$) from the control (saline).

Table 4. Cerebral tryptophan hydroxylase after chronic treatment with α-mephe or p-clphe without or with phe

Treatment	Tryptophan hydroxylase (units/g midbrain)
Saline	96.8 ± 17.0 (14)
α-Mephe	53.7 ± 12.3* (9)
α-Mephe + phe	86.0 ± 22.0 (12)
p-Clphe	18.4 ± 8.3* (6)
p-Clphe + phe	33.6 ± 2.2* (6)

The measurements on 8 to 9-day-old rats were done 4–6 hr after the last of three injections (one per day) of standard doses of the indicated substances.

* Significant difference ($P < 0.01$) from the saline group.

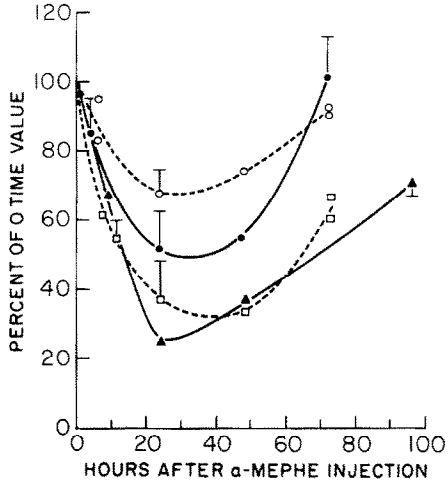

Fig. 1. Time course of change in cerebral indole variables and in hepatic phenylalanine hydroxylase after a single injection of α-mephe. Midbrain TPH (●), 5-HT (○--○) and 5-HIAA (□—□) were determined in 6 to 8-day-old rats at the indicated hours after an injection of a standard amount (see Materials and Methods) of α-mephe; results refer to single animals or are means (bar = one SD) of results on three to fourteen animals. Triangles refer to previously published data [6] on hepatic phenylalanine hydroxylase in 6- to 8-day-old rats given the same injections of α-mephe.

concentration in the TPH assay mixture containing samples of the brain homogenate would be only about 0.024 mM. This amount of α-mephe would not be inhibitory (cf. Fig. 2), especially since the assays of TPH for Fig. 1 were carried out under saturating substrate (and cofactor) concentrations. Moreover, the inhibition *in vitro* was apparent immediately upon the addition of α-mephe, whereas that *in vivo* was appreciable 24 hr after its injection only, and recovery occurred several days later. The similarly slow loss and recovery of 5-HT after an injection of p-clphe (not shown) confirmed previous studies [23] on brain stem which also demonstrated that changes in 5-HT parallel in time the gradual suppression and slow recovery of TPH. Since this time course of change was similar to that in Fig. 1, the mechanism underlying the response to α-mephe may be the same as that to p-clphe. However, a salient difference was that treatment with p-clphe plus phe had the same effect as that with p-clphe alone, whereas simultaneous administration of phe prevented α-mephe from reducing the cerebral TPH level *in vivo* (Table 4). These results, together with those in Table 3, indicate that 5-HT depletion by p-clphe plus phe (as by p-clphe alone) was due to the suppression of TPH, whereas this suppression played no role in the 5-HT depletion of animals treated with α-mephe plus phe.

DISCUSSION

Studies of the modes of action of synthetic substances like p-clphe of α-mephe provide some insights into the various mechanisms whereby derangements of aromatic amino acid metabolism can arise. One of these mechanisms is exemplified by the loss of the hepatic phenylalanine hydroxylase activity after an injection of p-clphe. Guroff [24] has shown that restoration of the activity, which takes 3–4 days, awaits resynthesis of the enzyme. The phenomenon, therefore, was termed "irreversible inhibition". However, no such inhibition occurs *in vitro*. Moreover, not only the recovery, but also the loss of activity *in vivo* is then found to be a slow process [25], further indicating that p-clphe does not act on preformed phenylalanine hydroxylase molecules and that we are dealing with a mechanism operative only during the dynamic state of turnover of the enzyme in the living cell [6, 25]. A decrease in activity brought about by this type of mechanism is referred to here as "suppression" in contradistinction to "inhibition" which is a more rapid effect and is also seen *in vitro*. Thus, both α-mephe and p-clphe are suppressors of phenylalanine hydroxylase, both are only competitive inhibitors of tyrosine hydroxylase [10, 26], and, as demonstrated here, α-mephe (as well as p-clphe [6, 27]) suppressed the cerebral TPH *in vivo* and can also act as a weak competitive inhibitor of this enzyme *in vitro*. While p-clphe and α-mephe are thus qualitatively similar in their actions on the three aromatic amino acid hydroxylases, this paper also shows that the roles they play in 5-HT depletion of animal models for PKU are different.

In animals treated with p-clphe plus phe, the brain concentration of the 5-HT precursor, tryptophan, was expected to be subnormal, since their plasma phe levels were high enough (as high as in PKU and in the "α-mephe model" of the disease) to monopolize the L-transport system and thus to minimize the uptake of tryptophan. The present measurements (Table 3) show, however, that cerebral tryptophan deficiency, which has been found to be present in the brain of PKUs [1], was not present in the "p-clphe model". The higher cerebral tryptophan levels found upon treatment with p-clphe alone than with p-clphe plus phe (Table 3) suggest that the usual inhibition of tryptophan transport was operative in the latter animals but that it was counterbalanced by an effect of p-clphe on cerebral tryptophan metabolism. The suppression of TPH may have resulted in a buildup of tryptophan, but whether the percent of tryptophan normally converted to 5-HT is large enough to explain such a buildup may be questioned. Another, more lively, explanation may be that the inhibition by p-clphe of brain protein synthesis [28] with consequent under-utilization of tryptophan is

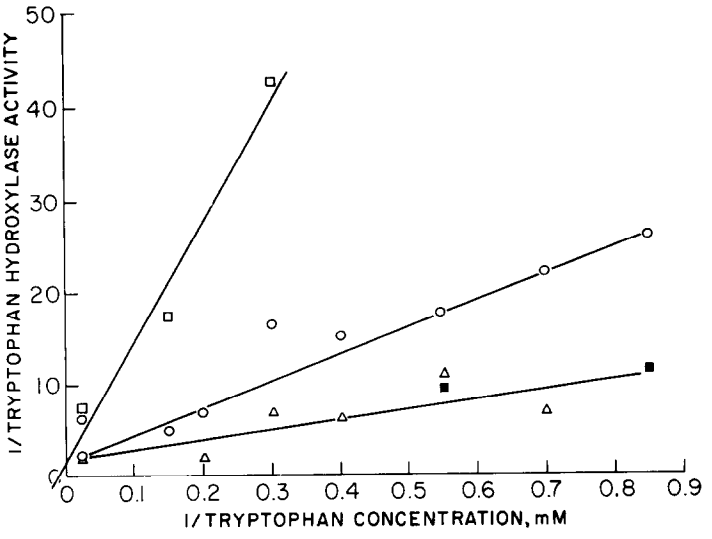

Fig. 2. Inhibition of tryptophan hydroxylase activity by addition of α-mephe *in vitro*. The points are single determinations on pools of midbrains from three normal rats, assayed as described under Materials and Methods except that various concentrations of tryptophan were used and added without (△) or with 0.58 (■), 2.8 (○) or 14.4 (□) mM α-mephe.

what counterbalanced the reduced uptake of this amino acid in these animals.

Several previous observations illustrate the principle that excess amounts of an amino acid may counteract the actions of a structural analogue by competing for enzymic targets or for transport systems or by causing metabolic alterations. For example, treatment with α-mephe causes a greater cerebral catecholamine depletion than does treatment with α-mephe plus phe [10] even though both substances are inhibitors of tyrosine hydroxylase. Similarly, concomitant phe administration entirely prevents the body growth inhibition and mortality caused by prolonged treatment of fetal rats with α-mephe alone (Brass and Greengard, unpublished). It has also been shown that large and frequent doses of phe partially counteract the suppression of hepatic phenylalanine hydroxylase by *p*-clphe [29] or α-mephe injections [6]. Such observations indicate that the problem of identifying artifacts resulting from treatment with synthetic (together with natural) amino acids is not necessarily solved if, as is customary, animals treated with that analogue alone are used as "controls". Indeed, one may arrive at erroneous conclusions. The suppression of TPH by α-mephe alone, for example, would suggest that this non-physiological mechanism is causing the 5-HT deficiency in the "α-mephe model" for PKU when, in fact, α-mephe (in contrast to *p*-clphe) did not suppress this enzyme in the presence of excess phe *in vivo* (Table 2). Thus, as in human PKU, hyperphenylalaninemia, with the resulting diminution of brain tryptophan content, accounts for the cerebral 5-HT deficiency in this model.

Rats that have been treated with α-mephe plus phe during the first 3 postnatal weeks exhibit an anomaly [8] (denoting exaggerated cerebral lateralization) in their spontaneous circling activity which implies diminished ability for spatial orientation [30] and probably underlies the maze learning debility and hyperactivity of the same animals [7, 8]. Since this circling activity is regulated by nigrostriatal dopaminergic pathways with the participation of serotoninergic ones [30], the anomaly implicates abnormal dopamine and 5-HT levels. The concentrations of these amines and also of the amino acids, however, revert to normal within about 2 days after the cessation of experimental hyperphenylalaninemia (i.e. after termination of the treatment on postnatal day 21), whereas the behavioral abnormalities are evident in adulthood. Similarly, PKUs who have been untreated during infancy remain mentally retarded even after subsequent dietary prevention of their hyperphenylalaninemia [2, 31]. To harmonize the permanence of the behavioral impairment with the reversibility of the neuroactive amine depletions one must assume that these depletions (if present during critical stages of development) can cause irreversible damage. Strong support for this assumption comes from currently emerging evidence for the important role of dopamine, norepinephrine and 5-HT in early brain development: each appears to promote the differentiation (in terms of morphology and function) of the specific neural structures that will depend on it as a transmitter later on [11, 12].

In connection with experimental (and perhaps also clinical) approaches to PKU, it is of interest that in infant rats treated with α-mephe plus phe concomitant tyrosine administration normalizes the cerebral concentration of catecholamines despite persisting hyperphenylalaninemia [10] and, similarly, tryptophan supplements restored the 5-HT deficit (Table 2). With supplementation of tyrosine or tryptophan or both, one could thus produce variants of this PKU model and, by comparing them for behavioral or neurostructural variables, determine whether (or which) lasting cerebral defects may be attributable to the deficiency in 5-HT, or catecholamines, or to alternative hyperphenylalaninemia-associated chemical insults during develop-

ment. To guard against all these potentially pathogenic factors, the current treatment of PKUs is aimed at maintaining normal blood phe levels; the minimal permissible phe intake, however, necessitates measures (a largely synthetic diet, prohibition of most normal foods, etc.) which lead to social trauma and frequent non-compliance with the treatment [32]. Supplementation with tryosine and tryptophan (in higher amounts than now recommended to merely assure normal growth) would mean added safety since, as indicated by the above-mentioned observations on the animal model, such supplementation can prevent the cerebral deficits in catecholamines and 5-HT even at elevated phe levels.

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