Modulation of cerebral catecholamine concentrations during hyperphenylalaninaemia

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Hyperphenylalaninaemia induced by daily injections of α -methylphenylalanine plus phenylalanine caused 20–40% decreases in cerebral dopamine (3,4-dihydroxyphenethylamine) and noradrenaline in 7- and 11-day-old rats. α -Methylphenylalanine alone as well as phenylalanine alone caused cerebral dopamine depletion. However, the effects were not additive, in that the depletion caused by α -methylphenylalanine was greater, not less, than that after treatment with both it and phenylalanine. Increased concentrations of tyrosine in the brain, owing to administered or endogenously formed tyrosine, could overcome the effect of excess phenylalanine on cerebral dopamine content. The fact that the inhibition of tyrosine hydroxylase by phenylalanine (or α -methylphenylalanine) *in vitro* was overcome by tyrosine concentrations similar to those effective *in vivo* further implicates the tyrosine hydroxylase inhibition as the mechanism underlying the dopamine depletion in hyperphenylalaninaemia. These results provide a theoretical basis for elevation, by tyrosine supplementation, of the cerebral phenylalanine/tyrosine ratio as a possible treatment modality for phenylketonuria.

Animal models of phenylketonuria have reproduced many of the changes that occur in the brain of human subjects with this disease, including drastic elevations of phenylalanine content (Greengard *et al.*, 1976; DelValle *et al.*, 1978), decreases in branched-chain amino acids (Lowden & LaRamee, 1969; Greengard *et al.*, 1979; Isaacs & Greengard, 1980; Lane *et al.*, 1980) and 5-hydroxytryptamine (serotonin) depletion (McKean *et al.*, 1968; Isaacs & Greengard, 1980; Lane *et al.*, 1980). However, the subnormal catecholamine concentrations in the brains of human phenylketonurics (McKean, 1972) are the one abnormality that has never been reported in any animal model of the disease. The present paper shows that there is in fact a deficit of cerebral catecholamines in a reasonably good rat model of phenylketonuria, and explains why this has not been noticed previously.

To achieve severe hyperphenylalaninaemia, infant rats were given a daily injection of phenylalanine plus α -methylphenylalanine, a suppressor of the hepatic phenylalanine hydroxylase activity *in vivo* (Greengard *et al.*, 1976). Adult rats that had been subjected to the same treatment during the first 3 postnatal weeks have previously been shown to exhibit an anomaly in the lateralization of their

circling activity, a behavioural pattern known to be regulated by dopaminergic, nigrostriatal pathways (Glick & Greengard, 1980). Studies on this and other models of phenylketonuria have revealed abnormalities in additional behavioural parameters, such as maze-learning ability and locomotor activity (Luttges & Gerren, 1979; Glick & Greengard, 1980; Lane *et al.*, 1980), which are also influenced by dopaminergic systems. In addition, monkey (Brozoski *et al.*, 1979; Simon *et al.*, 1980) and rat (Kempf *et al.*, 1974) experiments unrelated to hyperphenylalaninaemia have directly linked higher mental functions to the cortical dopamine (3,4-dihydroxyphenethylamine) and noradrenaline concentrations. Delineation of the manner in which phenylalanine and tyrosine regulate the amount of these neurotransmitters in the developing brain may pave the way to an understanding of the pathogenesis of mental retardation in phenylketonuria.

Experimental

Animals

Adult CDF Fischer rats (Charles River Breeding Laboratories, Wilmington, MA, U.S.A.) were time mated and parturition was confirmed within 12 h. Animals receiving chronic drug treatments were given daily subcutaneous injections of DL- α -methyl-

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phenylalanine (2.4 μ mol/g body wt.) plus phenylalanine (5.2 μ mol/g body wt.) or α -methylphenylalanine (2.4 μ mol/g) only, beginning on day 2 after birth until the day they were killed. Control littermates received daily injections of the 0.9% NaCl vehicle. Rats were decapitated at the indicated time after their last injection. Their brains (except olfactory bulbs) were immediately excised, weighed and placed in acid (see below).

Materials

DL- α -Methylphenylalanine, 6,7-dimethyl-5,6,7,8-tetrahydropteridine hydrochloride, catalase (EC 1.11.1.6 from bovine liver), pargyline hydrochloride and other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). LK6DF (20 cm $\times$ 20 cm, 0.250 mm-thick silica) t.l.c. plates were purchased from Whatman (Clifton, NJ, U.S.A.), and analytical-grade cation-exchange resin AG 50W-X8 (200–400 mesh; H⁺ form) was obtained from Bio-Rad Laboratories (Richmond, CA, U.S.A.). Catechol *O*-methyltransferase (EC 2.1.1.6) was prepared by the method of Axelrod & Tomchick (1958) as described by Coyle & Henry (1973). L-[ring-3,5-³H]Tyrosine (50 Ci/mmol; New England Nuclear Corp., Boston, MA, U.S.A.) was purified through a column (0.5 cm $\times$ 3 cm) of cation-exchange resin (Bio-Rad AG 50W-X8), freeze-dried and frozen for up to 1 month before use.

Assays

Catecholamines. Brains were homogenized in 1.5–2 vol. of 0.2 M-HClO₄ containing 10 mM-dithiothreitol, the homogenates were centrifuged at 7000 g for 10 min at 4°C and the supernatants were stored under N₂ at –20°C for less than 6 weeks. Assays were performed by a modification of the method of Coyle & Henry (1973), by using the technique of Saller & Zigmond (1978) to separate the methylated catecholamines. Blanks were prepared by substituting 40 mM-CaCl₂ for the MgCl₂ to inhibit the catechol *O*-methyltransferase. Internal standards were prepared by adding 1–1.5 ng of dopamine and noradrenaline to tubes containing the usual reaction mixture including brain homogenate. Each brain homogenate was assayed three times and the average value used. Calculations of dopamine and noradrenaline content were based on the calculation of the radioactivity (c.p.m.) recovered from known internal standards from several reaction tubes. This 'recovery' or variation in methylation of catecholamine standards was the same for the different brain homogenates used in this study.

Amino acids. Brains used for phenylalanine and tyrosine analysis were homogenized in 2 or 4 vol. of trichloroacetic acid (final concn. 0.3 M) and centrifuged for 20 min at 105 000 g. Phenylalanine was measured in the brain supernatants fluorimetrically

by the method of McCaman & Robins (1962) as modified by Faulkner (1965). Tyrosine was assayed by a modification (McGee *et al.*, 1972) of the spectrophotometric assay of Udenfriend & Cooper (1952). The assay for tyrosine was not altered by high concentrations of phenylalanine, and neither tyrosine nor phenylalanine assays were affected by the addition of 500 nmol of α -methylphenylalanine.

Tyrosine hydroxylase. For the measurement of tyrosine hydroxylase activities, striata were removed from several rat brains by the method of Glowinski & Iversen (1966), pooled, and homogenized in 9 vol. of cold 0.32 M-sucrose containing 0.6% Triton X-100. Assay was by a modification of the method of Nagatsu *et al.* (1964), except that we used higher concentrations of NaCl (280 mM) and Fe(NH₄)₂SO₄·6H₂O (10 mM), and 100 μ l of striata homogenate was incubated for 8 min at 37°C in a shaking water bath. Blanks were identical except for the omission of the synthetic cofactor 6,7-dimethyl-5,6,7,8-tetrahydropteridine. Under these experimental conditions, utilizing saturating (0.5 mM-tyrosine) substrate concentrations, the reaction was linear for more than 10 min and over a range of 1–10 mg of tissue homogenate.

Results

Changes in the brain catecholamine concentrations of suckling rats exposed to experimental phenylketonuria (Greengard *et al.*, 1976; DelValle *et al.*, 1978) were studied. The animals given this treatment from 2 days of age showed decreased cerebral catecholamines when killed 5–7 h after their last injection. Cerebral dopamine was significantly decreased by 27% and 34% in 7- and 11-day-old rats respectively, but showed no significant deficit in 16-day-old animals. Noradrenaline was decreased by 37% of control values on day 7, and showed a 27% decrease on day 16 (Table 1).

Cerebral catecholamine concentrations were also analysed according to sex. At 11 and 16 days no sex differences were seen. However, at 7 days of age male rats had higher concentrations of both dopamine (22% higher, $P < 0.01$) and noradrenaline (27% higher, $P < 0.01$; Table 1). Relative catecholamine depletion was equivalent in both sexes; male and female pups had 27% depletions of their basal (control) dopamine contents, and 34 and 39% decreases respectively in their noradrenaline contents. But the absolute catecholamine concentrations in the female hyperphenylalaninaemic rats, owing to their lower basal values, were lower than in their male littermates. To simplify the interpretation of data and decrease the number of variables, all subsequent experiments described in this paper were conducted on 7-day-old female rats.

Table 1. *Effect of chronic hyperphenylalaninaemia on cerebral catecholamines*

Animals were injected daily (subcutaneously) with α -methylphenylalanine (2.4 μ mol/g body wt.) plus phenylalanine (5.2 μ mol/g body wt.) or 0.9% NaCl (controls) from postnatal day 2 until the day of death. At 5–7 h after the last injection animals were decapitated and cerebral dopamine and noradrenaline measured as described in the Experimental section. All values are expressed as ng of catecholamine/g wet wt. of brain, and are means $\pm$ s.d. for the numbers of animals in parentheses. ^a $P < 0.01$ for difference from control female; ^b $P < 0.01$ for difference from corresponding females; ^c $P < 0.01$ for difference from controls; ^d $P < 0.025$ for difference from control.

Age (days)	Catecholamine	Control		Experimental	
		Male	Female	Male	Female
7	Dopamine	403 $\pm$ 113 (6) ^a	330 $\pm$ 47 (6)	293 $\pm$ 30 (7) ^{b,c}	242 $\pm$ 65 (6) ^c
	Noradrenaline	336 $\pm$ 12 (12)	501 $\pm$ 58 (3) ^b	269 $\pm$ 84 ^c (13)	393 $\pm$ 24 (4)
9	Dopamine	660	464	317	439
	Noradrenaline	746 $\pm$ 151 (3)	897 $\pm$ 203 (3)	632 $\pm$ 55 (4)	597 $\pm$ 26 (2)
11	Dopamine	882 $\pm$ 180 (6)	637 $\pm$ 104 (9)	611 $\pm$ 52 (6) ^c	604 $\pm$ 157 (10)
	Noradrenaline	592 $\pm$ 135 (4)	667 $\pm$ 78 (6)	597 $\pm$ 200 (5)	611 $\pm$ 122 (5)
16	Dopamine	506 $\pm$ 149 (4)	607 $\pm$ 149 (6)	386 $\pm$ 202 (5)	446 $\pm$ 66 (5)
	Noradrenaline	567 $\pm$ 150 (10)	574	416 $\pm$ 145 (10) ^d	444
18	Dopamine	543	699	574	444

The time course of cerebral catecholamine depletion was studied by measurements at 6, 12 and 26 h after the last injection of phenylalanine plus α -methylphenylalanine. Dopamine concentrations were lowest at 6 h, and by 12 h had almost returned to control values (Fig. 1). This contrasts markedly with the dopamine depletion observed when these rats were injected with α -methylphenylalanine alone. At 6 h after the last injection, cerebral dopamine concentrations were only 50% of control values, and at 18 h they were still only 77% of normal. With a single injection of α -methylphenylalanine the dopamine content was decreased to 64%, 50% and 66% of control values at 3, 6 and 12 h after injection. Thus α -methylphenylalanine itself caused a more severe and longer-lasting dopamine depletion than did injection of α -methylphenylalanine plus phenylalanine. However, the effect of α -methylphenylalanine alone also appears to dissipate by 24 h. The fact that the depletion (at 6 h) is not greater in the chronic experiments than in the acute experiments (i.e. 6 h after an injection to previously untreated rats) also indicates a lack of cumulative effect.

Since our model of phenylketonuria includes the use of α -methylphenylalanine, itself a potent depletor of cerebral catecholamines, the relationship between increases in phenylalanine (as seen in phenylketonuria) and catecholamine loss was unclear. Thus we decided to investigate the role of high phenylalanine itself in depleting catecholamines in our model system. However, one injection of phenylalanine alone will not sustain high increases in plasma or brain phenylalanine for many hours, since it is

Fig. 1. *Time course of dopamine depletion in response to treatment with phenylalanine plus α -methylphenylalanine or α -methylphenylalanine alone*

Female rats were injected with phenylalanine (5.2 μ mol/g body wt.) plus α -methylphenylalanine (2.4 μ mol/g) or α -methylphenylalanine only (2.4 μ mol/g) daily from day 2 of life (chronic) or on day 7 only (acute). Animals were killed on day 7 at the indicated hour after their last injection. Cerebral dopamine was measured as described in the Experimental section, and is expressed as a percentage ($\pm$ S.E.M.) of dopamine content of control (saline-injected) littermates killed at the same time. Each point represents the mean value for four to six rats. O, α -Methylphenylalanine plus phenylalanine (chronic); Δ , α -methylphenylalanine only (chronic); $\square$, α -methylphenylalanine only (acute).

rapidly metabolized by the hepatic phenylalanine hydroxylase. Therefore we injected α -methylphenylalanine alone, and 1 day later, when the hepatic phenylalanine hydroxylase was suppressed (Del-Valle *et al.*, 1978) but the cerebral dopamine was no longer depleted (Fig. 1), we injected 5 μ mol of phenylalanine/g. This resulted in an increase in brain phenylalanine to 1400 nmol/g at 6 h, but no depletion of cerebral dopamine (Table 2). However, injection of 12.5 μ mol of phenylalanine/g into animals with similarly inhibited hepatic phenylalanine hydroxylase increased brain phenylalanine to 2900 nmol/g at 6 h and caused a 24% deficit in cerebral dopamine. This increase in brain phenylalanine at 6 h was similar to that (2600 nmol/g) seen in the brains of rats chronically injected with α -methylphenylalanine and phenylalanine, and the cerebral dopamine decrease (27%) was also similar (cf. Table 3).

When three doses of phenylalanine (enough to increase cerebral phenylalanine to 2788 nmol/g at 6 h) were given without a prior injection of α -methylphenylalanine (line 5, Table 2), no catecholamine depletion occurred. However, since the hepatic phenylalanine hydroxylase was not inhibited in these animals, much of the phenylalanine was metabolized to tyrosine, causing an increase in cerebral tyrosine (from a control value of 65 nmol/g) to 400 nmol/g. To obtain similarly high cerebral tyrosine concentrations in rats with inhibited phenylalanine hydroxylase, rats were given 5 μ mol of tyrosine/g along with 5 μ mol of phenylalanine/g 1 day after treatment with α -methylphenylalanine. Brain phenylalanine was markedly increased in these animals, as was cerebral tyrosine, and once again cerebral dopamine concentrations remained unchanged (line 4, Table 2).

One possible explanation for the apparent reversal by tyrosine of the cerebral catecholamine depletion accompanying hyperphenylalaninaemia may be that the phenylalanine acts as a competitive inhibitor of tyrosine hydroxylase, and additional tyrosine overcomes this inhibition. To approach this question, tyrosine hydroxylase activity in striatal homogenates was measured *in vitro* with various concentrations of phenylalanine and tyrosine. A double-reciprocal plot of the data for these experiments (Fig. 2) shows that phenylalanine does act as a competitive inhibitor ($K_i = 2.19 \times 10^{-4}$ M) of striatal tyrosine hydroxylase. It is clear that at least *in vitro* one can overcome phenylalanine inhibition of tyrosine hydroxylase by raising the tyrosine concentrations above the value *in vivo*. For example, tyrosine hydroxylase activity was 7.59 μ mol/min per g with a tyrosine concentration of 50 μ M (the approximate value *in vivo*), but only 1.00 when 2.4 mM-phenylalanine (the value seen in phenylketonuria and in our animal model of the disease) was added to the incubation. However, when the tyrosine concentration was raised to 0.5 mM, tyrosine hydroxylase activity in the presence of 2.4 mM-phenylalanine was 8.47 μ mol/min per g. This value is similar to the tyrosine hydroxylase activity *in vitro* measured with tyrosine concentrations found *in vivo* (in the absence of excess phenylalanine).

As previously reported by Udenfriend *et al.* (1965), α -methylphenylalanine is a competitive inhibitor of tyrosine hydroxylase *in vitro*. From the present double reciprocal plots of activity against the various tyrosine and α -methylphenylalanine concentrations used, the K_i of α -methylphenylalanine (1.46×10^{-3} M) is an order of magnitude greater than that for phenylalanine (2.19×10^{-4} M). Nonetheless, α -methylphenylalanine was found to be a more potent

Table 2. Catecholamine depletion in response to various cerebral concentrations of phenylalanine and tyrosine. Rats in groups 2 (second line) and 3 (third line) received 5.0 and 12.5 μ mol of phenylalanine/g body wt. respectively. Rats in group 4 (fourth line) were injected with 5 μ mol of phenylalanine/g plus 5 μ mol of tyrosine/g. All of these animals (groups 1–4) had their liver phenylalanine hydroxylase activity decreased by prior treatment with α -methylphenylalanine. The fifth group of rats received 13 μ mol of phenylalanine/g administered in three doses, first 5 μ mol/g followed 1 h later by 3.9 μ mol/g and another 3.9 μ mol/g 4 h after that. All rats were 7-day-old females which were killed 6–7 h after their first injection of phenylalanine. Catecholamine concentrations are expressed as percentages [$\pm$ s.d. (*n*)] of those of control (NaCl-injected) littermates treated and assayed at the same time. Phenylalanine and tyrosine concentrations [nmol/g of brain; $\pm$ s.d. (*n*)] were determined in the brains of a separate group of animals treated similarly to those in which cerebral catecholamines were assayed.

Hepatic phenylalanine hydroxylase	Treatment (μ mol/g)		Brain amino acids (nmol/g)		Brain catecholamines (% of control)	
	Phenylalanine	Tyrosine	Phenylalanine	Tyrosine	Dopamine	Noradrenaline
Inhibited	—	—	85 $\pm$ 12 (4)	63 $\pm$ 17 (3)	108 $\pm$ 10 (4)	—
Inhibited	5	—	1268, 1668	87 $\pm$ 23 (4)	100 $\pm$ 15 (4)	86 $\pm$ 18 (4)
Inhibited	12.5	—	2900 $\pm$ 303 (6)	94 $\pm$ 34 (3)	76 $\pm$ 14 (3)	90 $\pm$ 19 (3)
Inhibited	5	5	3935 $\pm$ 930 (4)	472 $\pm$ 219 (4)	104 $\pm$ 11 (3)	116 $\pm$ 16 (3)
Normal	13	—	2788 $\pm$ 236 (4)	400 $\pm$ 135 (4)	113 $\pm$ 27 (6)	86 $\pm$ 21 (6)

Table 3. Comparison of the dopamine-depleting effects of α -methylphenylalanine with that of phenylalanine and of α -methylphenylalanine plus phenylalanine

Female rats (7 days old) with decreased phenylalanine hydroxylase activity were injected with 0.9% NaCl (line 1), phenylalanine alone (12.5 μ mol/g, line 2), α -methylphenylalanine (2.4 μ mol/g, line 3), or α -methylphenylalanine (2.4 μ mol/g) plus phenylalanine (5.2 μ mol/g, line 4). At 5–7 h after injection their brains were removed and cerebral dopamine concentrations determined in relation to those of saline-injected animals (% of control). Similarly treated animals were used to determine the cerebral phenylalanine and tyrosine contents of animals undergoing these treatments. Values represent means $\pm$ s.d. (n).

Treatment	Phenylalanine	Tyrosine	Dopamine (% of control)
Saline	80 $\pm$ 17 (5)	77 $\pm$ 13 (3)	100 $\pm$ 14 (6)
Phenylalanine	3194 $\pm$ 323 (4)	94 $\pm$ 34 (3)	76 $\pm$ 14 (3)
α -Methylphenylalanine	269 $\pm$ 32 (3)	82 $\pm$ 11 (3)	50 $\pm$ 12 (6)
α -Methylphenylalanine plus phenylalanine	2620 $\pm$ 420 (6)	120 $\pm$ 27 (4)	73 $\pm$ 20 (6)

Fig. 2. Double-reciprocal plot of tyrosine concentration against the rate of tyrosine hydroxylation with various concentrations of phenylalanine

The standard incubation mixture plus various concentrations of phenylalanine was used (○, 0 mM; △, 0.6 mM; □, 1.2 mM; ▲, 2.4 mM; ■, 3.6 mM). Enzyme was from a pool of the striatum of several adult rats. Assays were performed in triplicate for each point and the average value was used. The K_i , as calculated from the curves whose slope and intercept values were significant (with $P < 0.05$), was 2.19×10^{-4} M.

differed with respect to cerebral tyrosine content, which was higher with the dual treatment than with α -methylphenylalanine alone [see column 2 ('Tyrosine'), Table 3]. The dopamine depletion accompanying the injection of α -methylphenylalanine plus phenylalanine was similar to that seen in rats (with lower phenylalanine hydroxylase activity) given enough phenylalanine to achieve similar elevations in cerebral phenylalanine (Table 3, lines 2 and 4).

Discussion

Subnormal concentrations of noradrenaline, adrenaline and their metabolites in the blood and urine of phenylketonurics provided the first indications for the association of this disease with altered catecholamine metabolism (Weil-Malherbe, 1955; Nadler & Hsia, 1961; Cession-Fossion *et al.*, 1966; Curtius *et al.*, 1972). However, this corollary of the human condition could apparently not be imitated in animal models of phenylketonuria, so that no experimental system was available to explore the various reasons put forward to explain this catecholamine depletion. Among these were: (a) high concentrations of phenylalanine inhibiting tyrosine hydroxylase, the rate-limiting enzyme in catecholamine biosynthesis (Levitt *et al.*, 1965), (b) subnormal tyrosine concentrations limiting the rate of the tyrosine hydroxylase reaction, and (c) metabolites of phenylalanine (arising from the transamination pathway) compromising catecholamine synthesis in some unspecified way.

Although theory (a) was based on evidence for the inhibition by phenylalanine of the tyrosine hydroxylase activity *in vitro*, further studies were required to determine whether or not this inhibition could entirely account for the hyperphenylalaninaemia-induced dopamine depletion *in vivo*. The present study appears to provide an affirmative answer. Possibility (b) can definitely be excluded, since the

depletor of cerebral catecholamines than was phenylalanine. Moreover, their effects were not additive in respect to catecholamine depletion *in vivo*; the depletion seen 6 h after injection of α -methylphenylalanine plus phenylalanine was less, rather than more, than that seen 6 h after injection of α -methylphenylalanine alone (Table 3, lines 3 and 4). They also

tyrosine concentrations in our hyperphenylalaninaemic rats were above rather than below normal. The assumption (c), that metabolites of phenylalanine are responsible for the catecholamine depletion, also appears to be superfluous. Tyrosine supplementation of the severely hyperphenylalaninaemic rats is unlikely to have prevented the formation of these metabolites, and yet it abolished the catecholamine deficit. It did so at tyrosine concentrations similar to those needed to overcome the effect of excess phenylalanine on the tyrosine hydroxylase activity *in vitro*. The observations described here thus provide some of the missing evidence in favour of the tyrosine hydroxylase-inhibition theory of catecholamine depletion of phenylketonuria.

Rat models of phenylketonuria showing no cerebral catecholamine deficits include Wistar rats rendered hyperphenylalaninaemic with essentially the same methods as used here (Lane *et al.*, 1980). One obvious explanation for this observation may be that brains were sampled at such late times after the daily injection of phenylalanine plus α -methylphenylalanine when the catecholamines were at near-normal concentrations (see Fig. 1). Another explanation, of more general importance, lies in the ease with which a relatively small increase in tyrosine can counteract the catecholamine-depleting effect of hyperphenylalaninaemia (Table 2, lines 3 and 4). Thus a less efficacious treatment, that is one in which the brain phenylalanine concentrations are lower or the cerebral tyrosine concentrations are somewhat higher than in our model of phenylketonuria, may not produce noticeable catecholamine depletion.

The failure of our treatment to decrease dopamine concentrations in the brains of 16-day-old rats may be rooted in this same phenomenon. The small rise in phenylalanine hydroxylase activity during the suckling period (McGee *et al.*, 1972) despite α -methylphenylalanine treatment (DelValle *et al.*, 1978), may result in a cerebral phenylalanine/tyrosine ratio in the 16-day-old rat too low to decrease significantly the rate of tyrosine hydroxylation. Another contributing factor may be that as the suckling rat increases its capacity to protect dopamine and noradrenaline in storage vesicles (Coyle & Axelrod, 1971; Kirksey *et al.*, 1978; Deskin *et al.*, 1981), the brain may become more capable of withstanding the temporary loss in synthetic potential imposed by each daily treatment.

Relevance to the treatment of phenylketonuria

In view of indications that cerebral catecholamine deficiency compromises higher intellectual functions (see the Introduction), this new evidence for the importance of tyrosine as well as phenylalanine

concentrations in modulating the dopamine content of the hyperphenylalaninaemic brain may be pertinent to the management of phenylketonuria. For it suggests that the severe cerebral catecholamine depletion experienced by phenylketonurics may be reversed by a dietary regimen including tyrosine supplementation. Since the dietary addition of an appropriate excess of tyrosine would be effective with only a moderate decrease in phenylalanine intake, this could be accomplished more easily than the present treatment of phenylketonuria, where minimal phenylalanine intake requires severe food limitations, which involves social trauma, frequent failures to comply with the treatment, and difficulty in balancing phenylalanine restriction against the amount required for optimal growth. It has been reported (Butler *et al.*, 1981) that children deficient in dihydropteridine reductase, and thus in catecholamines and 5-hydroxytryptamine, benefit from treatment with precursors of these neuroactive amines. Thus the concept of treating cerebral amine deficiency with precursor administration is not new, although the present paper is the first to suggest the possible usefulness of such an approach to correcting some of the cerebral abnormalities associated with phenylketonuria.

α -Methylphenylalanine as an experimental tool

Suppression of phenylalanine hydroxylase *in vivo* with an injection of α -methylphenylalanine (without or with phenylalanine) is a slow, irreversible process. Minimal activity is attained in 20 h, and only after an interval (about 2 days) required for the resynthesis of the enzyme does the activity return to normal (DelValle *et al.*, 1978). Thus an advantage of using this agent to imitate phenylketonuria is that administration once in 2 days is sufficient to maintain steadily low phenylalanine hydroxylase activity *in vivo*.

The inhibition by α -methylphenylalanine of tyrosine hydroxylase, on the other hand, is rapid and reversible *in vitro*, and the catecholamine depletion *in vivo* begins to be restored within a few hours (Fig. 1). There was a time interval after α -methylphenylalanine administration when the dopamine concentrations had returned to normal but the hepatic phenylalanine hydroxylase, and thus the elimination of injected phenylalanine, was still suppressed. Thus the catecholamine deficit ensuing this injection is attributable to the phenylalanine itself. These results are in accord with the fact that phenylalanine as well as its α -methyl analogue are effective inhibitors of tyrosine hydroxylase *in vitro*. However, the magnitude of the dopamine depletion was greater in response to α -methylphenylalanine alone than to α -methylphenylalanine plus phenylalanine. One explanation for this is that the small increase in tyrosine associated with the combined treatment is

enough to preclude the inhibition of tyrosine hydroxylase by α -methylphenylalanine, and thus mask the depleting effect of α -methylphenylalanine. Another possibility is that excess phenylalanine blocks the transport of α -methylphenylalanine into the brain. In either case, a general, practical implication of the observations summarized in Table 3 is that, since elevated concentrations of an amino acid can minimize the side effects of synthetic analogues, study of the organism treated with such an analogue alone does not predict the extent to which these side effects will in fact be operative in animals treated with both substances.

Rats given α -methylphenylalanine alone therefore cannot be considered as 'controls' for those which, injected with phenylalanine as well, were meant to imitate the human disease of phenylketonuria. Instead, they can be looked upon as a new experimental system for research on aberrant dopaminergic functions associated with several conditions, including the minimal-brain-damage syndrome in children. The functional consequences of the reversible dopamine depletion or of prolonged depletion maintained with chronic α -methylphenylalanine treatment are not known. The obvious potential of this system to provide new insights comes from the fact that the mechanism underlying the experimental abnormality in catecholamine metabolism is clearly different from that in currently used models, where this is achieved through irreversible destruction of particular brain structures by surgical lesion or by administration of dopamine analogues (e.g. 5-hydroxydopamine) which destroy catecholaminergic neurons.

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