The effects of chronic hyperphenylalaninaemia on mouse brain protein synthesis can be prevented by other amino acids

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A prolonged elevation in the concentrations of circulating phenylalanine was maintained in newborn mice by daily injections of phenylalanine and a phenylalanine hydroxylase inhibitor, α -methylphenylalanine. The result of this chronic hyperphenylalaninaemia was an accumulation of vacant or inactive monoribosomes that persisted for 18 h of each day. An elongation assay *in vitro* with brain postmitochondrial supernatants demonstrated that, in addition, there was an equally prolonged decrease in the rates of polypeptide-chain elongation by the remaining brain polyribosomes. Analyses of the free amino acid composition in the brains of hyperphenylalaninaemic mice showed a loss of several amino acids from the brain, particularly the large, neutral amino acids, which are co- or counter-transported across plasma membranes with phenylalanine. When a mixture of these amino acids (leucine, isoleucine, valine, threonine, tryptophan, tyrosine, methionine) was injected into hyperphenylalaninaemic mice, there was an immediate cessation of monoribosome accumulation in the brain and there was no inhibition of the rates of polypeptide-chain elongation. Although the concentrations of the large, neutral amino acids in the brain were partially preserved by treatment of hyperphenylalaninaemic mice with the amino acid mixture, the elevated concentrations of phenylalanine remained unaltered. The amino acid mixture had no detectable effect on brain protein synthesis in the absence of the hyperphenylalaninaemic condition.

Almost all inborn errors of amino acid metabolism result in severe and often devastating neurological disorders. Phenylketonuria is the most common of these genetic diseases, and if untreated leads to severe mental retardation. The disease results from a non-functional hepatic enzyme, phenylalanine hydroxylase, which normally converts 90% of all dietary phenylalanine into tyrosine. The concentration of circulating phenylalanine in untreated patients with phenylketonuria is elevated 20–40-fold above normal. Despite the extensive biochemical characterization of phenylketonuria, the mechanism by which these elevated concentrations of phenylalanine interrupt normal brain development in human infants still is not understood.

Reports from this and other laboratories have shown that a single administration of phenylalanine (1–2 mg/g body wt.) into newborn animals produces a brief 10–20-fold elevation in the concentrations of phenylalanine in the plasma and brain (McKean *et al.*, 1968; Siegel *et al.*, 1971; Roberts &

Morelos, 1976; Binek *et al.*, 1981) and a concomitant loss of several amino acids from the brain (McKean *et al.*, 1968; Cohen & Lajtha, 1972; Roberts & Morelos, 1976; Hughes & Johnson, 1976). The amino acids that are particularly affected by this acute hyperphenylalaninaemia are the large, neutral amino acids, which are believed to be co- or counter-transported across the plasma membrane with phenylalanine. These amino acids are leucine, isoleucine, valine, threonine, tryptophan, tyrosine and methionine. While liver protein metabolism remains unaltered (Aoki & Siegel, 1970; MacInnes & Schlesinger, 1971), acute hyperphenylalaninaemia causes a transient inhibition of brain protein synthesis (Aoki & Siegel, 1970; MacInnes & Schlesinger, 1971; Taub & Johnson, 1975; Hughes & Johnson, 1978), which appears to result from decreased rates of initiation (Taub & Johnson, 1975) and polypeptide-chain elongation (Hughes & Johnson, 1978). The loss of amino acids from the brain has been implicated by several investigators as the cause of the inhibition of brain protein synthesis during acute hyperphenylalaninaemia (Aoki & Siegel, 1970; Geison & Siegel, 1975; Hughes &

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Johnson, 1976; Berger *et al.*, 1979). To provide further evidence for this hypothesis, Hughes & Johnson (1978) showed that treatment of acutely hyperphenylalaninaemic mice with a mixture of the large, neutral amino acids prevented the inhibition of brain protein synthesis, even in the presence of a 70-fold excess of phenylalanine in the brain.

Chronic hyperphenylalaninaemia, produced by daily injections of phenylalanine and the phenylalanine hydroxylase inhibitor, α -methylphenylalanine, has the same effect on brain protein synthesis in newborn mice as does acute hyperphenylalaninaemia; that is, it inhibits the rates of initiation and elongation (Binek *et al.*, 1981). However, since phenylalanine hydroxylase activity is blocked continuously in these animals, high concentrations of phenylalanine are maintained for most of the 24 h between injections, with no significant increase in tyrosine (Greengard *et al.*, 1976; DelValle *et al.*, 1978; Binek *et al.*, 1981). As a result of the prolonged elevation in phenylalanine concentrations, brain protein synthesis remained inhibited for as long as 18 h of each day. The consequences of chronic hyperphenylalaninaemia, when maintained throughout postnatal growth, are decreased myelination in the mature central nervous system (Johnson & Shah, 1980; Figlewicz & Druse, 1980) and a permanent learning impairment (Greengard *et al.*, 1979; Luttges & Gerren, 1980).

Whether decreased rates of neutral protein synthesis are responsible for the myelin deficits and/or permanent learning impairment remains speculative. That hypothesis can be tested, however, provided that an appropriate amino acid mixture can be used to circumvent the inhibition of protein synthesis associated with chronic hyperphenylalaninaemia. To that end, we examined the ability of a mixture of large, neutral amino acids to prevent the inhibition of brain protein synthesis during chronic hyperphenylalaninaemia.

Materials and methods

Materials

All L-amino acids, α -methyl-DL-phenylalanine, ATP, GTP, phosphoenolpyruvate and pyruvate kinase were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Ribonuclease-free sucrose was obtained from Schwarz/Mann, Orangeburg, NY, U.S.A., and Sephadex G-200 from Pharmacia Fine Chemicals, Uppsala, Sweden. L-[4,5- 3 H]Lysine (75 Ci/mmol) was purchased from Amersham/Searle, Arlington Heights, IL, U.S.A.; 3a70B preblended scintillation cocktail was from Research Products International, Elk Grove Village, IL, U.S.A.; and Celotape EH filters (0.5 μ m pore size) were from Millipore Corp., Bedford, MA, U.S.A.

Administration of amino acids

L-Phenylalanine (30 mg/ml) and α -methyl-DL-phenylalanine (6.45 mg/ml) were dissolved together in 0.15 M-NaCl (saline) and injected intraperitoneally into 3–5-day-old mice at 2 mg of phenylalanine and 0.43 mg of α -methylphenylalanine/g body wt. (67 μ l/g). In all experiments, mice received two such injections, 24 h apart, and control mice received two injections of the same volume of saline. The amino acid mixture contained L-valine, L-leucine, L-isoleucine, L-threonine, L-tyrosine, L-tryptophan and L-methionine dissolved in 0.15 M-NaCl/0.5 M-HCl (1.5 mg of each amino acid/ml). At 1 h after the second injection of α -methylphenylalanine and phenylalanine, the amino acid mixture was injected intraperitoneally at a concentration of 0.075 mg of each amino acid/g body wt. (50 μ l/g). Control animals for these injections received equivalent volumes of acidified saline. All solutions were warmed to 37°C before injection.

Sucrose-gradient centrifugation of brain polyribosomes and ribosomal subunits

Polyribosomes were isolated from whole brain homogenates in the presence of the ribonuclease inhibitor bentonite (1 mg/ml), by the procedure of Taub & Johnson (1975). The enriched polyribosome fraction was layered ($2.0 \pm 0.05 A_{254}$ units) on a 5.0 ml linear (0.7–1.5 M) sucrose gradient in TKM buffer (50 mM-Tris/HCl, pH 7.2, 25 mM-KCl, 5 mM-MgCl₂) and centrifuged at 190 000 g for 90 min in a Beckman SW50.1 rotor at 4°C. Sucrose gradients were analysed for A_{254} as described by Hughes & Johnson (1978).

The relative proportion of inactive ribosomes in the brain was determined by their dissociation with high salt. Enriched polyribosome fractions were adjusted with 3 M-KCl to a final concentration of 0.5 M-KCl, layered on 5.0 ml linear (0.7–1.5 M) sucrose gradients in TKM/0.5 M-KCl, and centrifuged at 100 000 g for 120 min.

Polypeptide-chain elongation by brain polyribosomes

Cell-free protein synthesis by isolated brain polyribosomes was performed essentially as described by Hughes & Johnson (1978). The concentration of protein in each brain postmitochondrial preparation was determined by analysis of its A_{260}/A_{280} ratio. Reaction mixtures (0.5 ml) consisted of 5 μ mol of Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid], pH 8.0, 25 μ mol of Tris, pH 8.0, 5 μ mol of KCl, 5 μ mol of MgCl₂, 20 μ g of pyruvate kinase, 12 μ mol of 2-mercaptoethanol, 5 μ mol of phosphoenolpyruvate, 2 μ mol of ATP, 0.12 μ mol of GTP, 4 μ Ci of [3 H]lysine, and 0.5 mg of protein from the brain postmitochondrial super-

natants. Triplicate reactions for each assay were terminated after 4, 8 and 12 min incubation at 37°C with 10% trichloroacetic acid, and the acid-insoluble material was collected on Celotrate EH filters.

Free amino acid analysis

After various injection regimens in newborn mice, whole brains were removed, pooled into sets of four brains each, and homogenized in 1 ml of TKM buffer. An equal volume of 10% trichloroacetic acid was added to the homogenate, and the acid-insoluble material was removed by centrifugation at 2000 g for 20 min. The free amino acids were extracted from the remaining acid-soluble fraction by a chloroform/methanol (1:2, v/v) wash and recovered in the upper phase. The samples were then frozen at -70°C and freeze-dried (to dryness) in a Lab-Conco Freeze Dry-3 apparatus. The freeze-dried material was analysed essentially as described by Spackman *et al.* (1958) in a Dionex micro-bore high-pressure single-column amino acid analyser with norleucine as the internal standard.

Results

Effect of the amino acid mixture on the accumulation of brain monoribosomes

Brain polyribosome-profile analyses after daily injections of α -methylphenylalanine and phenylalanine in newborn mice revealed a rapid accumulation of monoribosomes and a simultaneous loss of polyribosomes. At 3 h, when accumulation was maximum, 30–33% of all brain ribosomes existed as monoribosomes (Fig. 1b), whereas in saline-injected control mice there was always 17–18% monoribosomes (Fig. 1a). To determine the nature and origin of the accumulated monoribosomes, brain polyribosome preparations were treated with 0.5 M-KCl. These relatively high salt concentrations dissociate into subunits only those ribosomes that are not bound to mRNA or peptidyl-tRNA (Belitsina & Spirin, 1970; Hamburger *et al.*, 1973) and thus can be used to distinguish between active and inactive monoribosomes (Martin & Hartwell, 1970). The number of active monoribosomes remaining after salt dissociation was identical in the brains of chronic hyperphenylalaninaemic mice (Fig. 1d) and saline-injected control mice (Fig. 1c). Therefore the monoribosomes that accumulated during chronic hyperphenylalaninaemia were not bound to mRNA or fragments of mRNA, and probably reflected decreased rates of protein-synthesis initiation.

The composition of the amino acid mixture used in this study was determined by the mixture's ability to prevent the accumulation of brain monoribosomes caused by injections of α -methylphenylalanine and phenylalanine. Several combinations of a

variety of amino acids and compositions showed that the most successful amino acid mixture for chronic hyperphenylalaninaemic mice was that developed by Hughes & Johnson (1977a,b) for acute hyperphenylalaninaemia. This mixture consisted of the seven large, neutral amino acids, which are thought to share the same transport carrier as phenylalanine: valine, leucine, isoleucine, threonine, tryptophan, tyrosine and methionine. Although tyrosine was an optional component of the amino acid mixture used for acute hyperphenylalaninaemia, it was essential for treatment of chronic hyperphenylalaninaemia (results not shown). This tyrosine requirement was not surprising, since the presence of α -methylphenylalanine in chronic hyperphenylalaninaemic mice inhibits tyrosine formation (Greengard *et al.*, 1976).

In order to monitor the preventative effects of this amino acid mixture on brain monoribosome accumulation during chronic hyperphenylalaninaemia, we analysed brain polyribosome profiles at several times before and after treatment with the amino acid mixture (Fig. 2). Under the control conditions in which the hyperphenylalaninaemic mice received acidified saline as their subsequent injection, monoribosome accumulation was maximum at 3 h, producing 34% monoribosomes. At the time that the amino acid mixture was administered to the hyperphenylalaninaemic mice, brain monoribosomes already had begun to accumulate, producing 24% monoribosomes. However, immediately after treatment with the amino acid mixture, further accumulation of monoribosomes was inhibited and complexes began to recover gradually (Fig. 2). Neither the amino acid mixture nor the acidified saline had any effect on brain polyribosome profiles in the absence of the chronic hyperphenylalaninaemic conditions (results not shown).

Effects of the amino acid mixture on polypeptide-chain elongation by brain polyribosomes

Because polyribosome-profile analysis does not always reveal the true state of protein-synthesis activity (Hughes & Johnson, 1978), brain polyribosome preparations from chronically hyperphenylalaninaemic mice were examined for their ability to support protein synthesis *in vitro*. The translational system used in this study does not allow the initiation of new polypeptide synthesis (Hughes & Johnson, 1978) and therefore provided a direct measurement of the rates of polypeptide-chain elongation. Because the concentration of free lysine in the brain is not affected by chronic hyperphenylalaninaemia (Isaacs & Greengard, 1980), [3 H]-lysine served as the radioactive precursor for the assay.

At 3 h after an injection of α -methylphenylalanine and phenylalanine, the rates of lysine incorporation

Fig. 1. *KCl* dissociation of monoribosomes that accumulate in the brains of mice after injections of α -methylphenylalanine

Newborn mice (3–5 days old) received two injections of either 0.43 mg of α -methylphenylalanine and 2 mg of phenylalanine/g body wt. (b, d) or an equivalent volume of saline (a, c), 24 h apart. At 3 h after the second injection, brains were removed and homogenized, and polyribosomes were recovered in TKM buffer. For (a) and (b), polyribosomes were resolved on a linear (0.7–1.5 M) sucrose gradient in TKM buffer as described in the Materials and methods section. For (c) and (d), KCl was added to the polyribosomes to a final concentration of 0.5 M and polyribosomes were separated on a linear (0.7–1.5 M) sucrose gradient in TKM/0.5 M-KCl as described in the Materials and methods section. The direction of sedimentation is from left to right.

by brain polyribosomes were 66% of the rates measured with brain polyribosomes from saline-injected control mice (Table 1). The increased number of inactive monoribosomes (Fig. 1) in the protein-synthesis assay prepared from hyperphenylalaninaemic mice could account for only a 12% decrease in the rates of lysine incorporation into protein. Therefore polypeptide-chain elongation, as

well as initiation events, must have been inhibited by the chronic hyperphenylalaninaemic conditions. When these hyperphenylalaninaemic mice were treated with the amino acid mixture 1 h after the second injection of α -methylphenylalanine and phenylalanine, the rates of lysine incorporation were 93% of the control rates. This small decrease in protein in protein synthesis was completely accoun-

Fig. 2. Effect of an amino acid mixture on monoribosome accumulation in the brains of hyperphenylalaninaemic mice

Neonatal mice (3–5 days old) received two injections of 0.43 mg of α -methylphenylalanine and 2 mg of phenylalanine/g body wt. 24 h apart. At 1 h after the second injection (arrow), mice received another injection of either 0.075 mg of each large, neutral amino acid/g body wt. (●), or an equivalent volume of acidified saline (○). At various times after the injections, brains were removed and homogenized in TKM buffer. Polyribosomes were prepared and resolved on linear sucrose gradients as described in the Materials and methods section. Results are expressed as $100 \times$ the ratio of the area under the monoribosome peak to the area under all ribosomal peaks. Each point represents the average of three or four independent determinations, each done in duplicate, $\pm$ S.E.M.

prolonged and perhaps more dramatic loss of the same amino acids from the brains of these mice.

Analysis of the free amino acid content in the brains of chronically hyperphenylalaninaemic mice supported our expectations. In accord with previous findings (Hughes & Johnson, 1976, 1977b), the large, neutral amino acids, which are co- or counter-transported with phenylalanine, were more affected by the high concentrations of phenylalanine than was any other class of amino acids. Fig. 3 shows the changes in the concentrations of four representative large, neutral amino acids: methionine, leucine, isoleucine and valine. The loss of these amino acids from the brain was greatest between 3 and 6 h after the injection with α -methylphenylalanine and phenylalanine. Coincidentally, phenylalanine concentrations in the brain were at their maximum during this period (Binek *et al.*, 1981). By 24 h after injection, when phenylalanine concentrations in the brain were almost back to control values, the concentrations of all other amino acids, except methionine, had completely recovered (Fig. 3).

The alterations in the concentrations of the amino acids not shown in Fig. 3 were very similar to the changes reported for acute hyperphenylalaninaemia (McKean *et al.*, 1968; Roberts & Morelos, 1976; Hughes & Johnson, 1976) and more recently for chronic hyperphenylalaninaemia in rats (Isaacs & Greengard, 1980; Dienel, 1981). The concentration of glycine, for example, increased temporarily in response to hyperphenylalaninaemia ($1.77 \mu\text{g}/\text{brain}$ in control mice; $2.29 \mu\text{g}/\text{brain}$ at 3 h in hyperphenylalaninaemic mice). There were very slight decreases in the other small, neutral amino acids (alanine, serine, proline, histidine) and in the acidic amino acids after the last injection of α -methylphenylalanine and phenylalanine. Again, complete recovery was not apparent until 24 h later. Finally, there were no alterations in the concentrations of the basic amino acids (lysine, arginine) throughout the hyperphenylalaninaemic condition.

To determine the ability of the amino acid mixture to alleviate the amino acid imbalance in the brain resulting from chronic hyperphenylalaninaemia, the mixture was injected 1 h after the last injection of α -methylphenylalanine and phenylalanine, and 2 h later brains were removed and prepared for free amino acid analyses. Treatment with the amino acid mixture partially, but not completely, prevented the loss of the large, neutral amino acids from the brain (Fig. 4). Even though equal amounts of the seven amino acids were injected, the mixture was more effective for some amino acids, such as isoleucine, methionine and valine, than for others, such as leucine. The amino acid mixture had no effect on the concentrations of those amino acids that were not included in the mixture (results not shown). The

ted for by the additional inactive monoribosomes still present at the time of the assay (Fig. 2).

Effects of the amino acid mixture of free amino acid content in the brain

In acute hyperphenylalaninaemia, a 10–20-fold increase in phenylalanine led to a brief loss of several other amino acids from the brain (McKean *et al.*, 1968; Cohen & Lajtha, 1972; Roberts & Morelos, 1976; Hughes & Johnson, 1976). Since there is a more persistent 30–40-fold increase in phenylalanine during chronic hyperphenylalaninaemia (Greenard *et al.*, 1976; DelValle *et al.*, 1978; Binek *et al.*, 1981), it was expected that there would be a more

Table 1. *Rates of polypeptide-chain elongation in the brains of hyperphenylalaninaemic mice after treatment with the amino acid mixture*

Neonatal mice (3–5 days old) received two initial injections, 24 h apart, of either 0.43 mg of α -methylphenylalanine (α -MePhe) and 2 mg of phenylalanine/g body wt., or an equivalent volume of saline. In some conditions, 1 h after the second injection of α -methylphenylalanine and phenylalanine mice received another injection of either 0.075 mg of each large, neutral amino acid/g body wt. or an equal volume of acidified saline. At 3 h the second injection of α -methylphenylalanine and phenylalanine, brains were removed and homogenized in TKM buffer. A post-mitochondrial supernatant (0.5 mg of protein) from the homogenate was added to a cell-free elongation assay containing [3 H]lysine as described in the Materials and methods section. Rates of lysine incorporation into acid-insoluble material were determined during the linear phase of the reaction (4–12 min). Results are means $\pm$ S.E.M. for the numbers of independent determinations shown in parentheses.

Initial injections	Subsequent injection	[3 H]Lysine incorporation	
		(mol/min)	(% of control)
Saline (control)	—	3.09 ± 0.20 (9)	100
α -MePhe + Phe	—	2.03 ± 0.14 (9)	66
α -MePhe + Phe	Acidified saline	2.10 ± 0.18 (5)	68
α -MePhe + Phe	Amino acids	2.86 ± 0.18 (5)	93

Fig. 3. *Effect of chronic hyperphenylalaninaemia on the concentrations of some large, neutral amino acids in the brain*

Mice (5 days old) received two injections, 24 h apart, of either 0.43 mg of α -methylphenylalanine and 2 mg of phenylalanine/g body wt. (●) or an equal volume of saline (○). At 3, 6, 12 or 24 h after the second injection, brains were removed and homogenized in TKM buffer. Free amino acids were extracted from the homogenate and analysed as described in the Materials and methods section. The large, neutral amino acids shown here are methionine (a), isoleucine (b), valine (c) and leucine (d). Each point represents the average amino acid concentration per brain of four or eight pooled brains.

Fig. 4. *Concentrations of some large, neutral amino acids in the hyperphenylalaninaemic brain after treatment with the amino acid mixture*

Mice (5 days old) received two injections, at 24 h intervals, of 0.43 mg of α -methylphenylalanine and 2 mg of phenylalanine/g body wt. At 1 h after the second injections of α -methylphenylalanine and phenylalanine, mice received a third injection of either 0.075 mg of each large, neutral amino acid per g body wt. (□) or an equivalent volume of acidified saline (■). Then 2 h later, brains were removed and homogenized in TKM buffer. Free amino acids were extracted from the homogenates and analysed as described in the Materials and methods section. The mice used as controls (■) received two injections of saline, 24 h apart, and their brains were removed 3 h after the second injection. Results are expressed as the average amino acid concentration per brain of two pools of four brains each.

phenylalanine concentrations in the brains of hyperphenylalaninaemic mice remained equally elevated whether they were treated with the acidified saline alone (20.6 times that in saline-injected control) or the amino acid mixture (21.2 times that in saline-injected control). Finally, a comparison of the amino acid analyses obtained from hyperphenyl-

alaninaemic mice that received no treatment and hyperphenylalaninaemic mice that received the acidified saline showed no significant differences for any amino acid (compare Fig. 3 and Fig. 4).

Discussion

Polyribosome disaggregation has been a popular term for describing the changes in brain polyribosome profiles that occur during experimental hyperphenylalaninaemia. However, the present study suggests that this is perhaps an erroneous or misleading term, since it allows the possibility that smaller polyribosomes (e.g. dimers, trimers) can be formed by the degradation of larger polyribosomes (Roberts & Morelos, 1976). We observed no increase in the number of small polyribosomes during chronic hyperphenylalaninaemia, and in fact the only size class of ribosomes that did increase in response to the excess phenylalanine was the monoribosome (Figs. 1*a* and 1*b*). Further analysis of brain monoribosomes by salt dissociation revealed that the accumulated monoribosomes were not associated with mRNA or fragments of mRNA (Figs. 1*c* and 1*d*). These results confirmed that the changes in polyribosome profiles were due to lower rates of protein-synthesis initiation and not to the degradation of polyribosome complexes. Therefore the phenylalanine-induced alterations in polyribosome profiles would be more aptly described as an accumulation of inactive monoribosomes, to emphasize that an inhibition of protein-synthesis initiation produced these changes.

An increase in the number of inactive monoribosomes demonstrated only that the rates of protein-synthesis initiation had been inhibited relative to the rates of polypeptide-chain elongation. However, it was possible that even elongation was influenced by the chronic hyperphenylalaninaemic condition. Direct measurements of the rates of polypeptide-chain elongation in a translation system *in vitro*, unable to support initiation, showed that elongation by the remaining active polyribosomes was inhibited during chronic hyperphenylalaninaemia (Table 1).

As in acute hyperphenylalaninaemia (McKean *et al.*, 1968; Roberts & Morelos, 1976; Hughes & Johnson, 1976), most amino acids were affected to some extent by the elevated phenylalanine concentrations, but the large neutral amino acids were the most sensitive to the chronic hyperphenylalaninaemic condition. Although other studies have reported a similar depletion of amino acids in the brain during chronic hyperphenylalaninaemia (Isaacs & Greengard, 1980; Dienel, 1981), the present study examined both the initial loss of the large, neutral amino acids and their recovery to normal values, which appeared to be coincident with

the recovery of near-normal phenylalanine concentrations (Fig. 3). Maximum decrease in the concentrations of the amino acids shown in Fig. 3 (methionine, leucine, isoleucine, valine) occurred between 3 and 6 h after injection of α -methylphenylalanine and phenylalanine, where they were on average only 30% (range = 23–37%) of the control values. In contrast with the acute hyperphenylalaninaemic conditions, maximum loss of the same amino acids occurred between 0.5 and 1 h after injection with phenylalanine alone, where they were approx. 70% (range = 54–89%) of the control values (McKean *et al.*, 1968; Roberts & Morelos, 1976). Therefore the amino acid imbalance in the brain produced by chronic hyperphenylalaninaemia is not only more pronounced than during acute hyperphenylalaninaemia, but also considerably more enduring.

When a mixture of the large, neutral amino acids was injected intraperitoneally into chronic hyperphenylalaninaemic mice, monoribosome accumulation in the brain ceased immediately, and after 1 h polyribosome profiles began to recover (Fig. 2). Apparently, the presence of the amino acid mixture blocked the phenylalanine-induced inhibition of protein-synthesis initiation. In addition, treatment of hyperphenylalaninaemic mice with the mixture of amino acids resulted in elongation rates that were 93% of the control values (Table 1). The additional free monoribosomes in the polyribosome preparations from the amino-acid-treated hyperphenylalaninaemic mice (Fig. 2, at 3 h) led to a slight decrease in lysine incorporation, and their contribution completely accounted for the persisting 8% inhibition of elongation rates. It appeared therefore that treatment of hyperphenylalaninaemic mice with a mixture of large, neutral amino acids completely blocked the inhibition of brain protein synthesis at the levels of initiation and elongation.

The ability of the amino acid mixture to prevent the inhibition of brain protein synthesis during acute and chronic hyperphenylalaninaemia suggests that the amino acid mixture maintained functional concentrations of the amino acids in the brain. The present study confirms this prediction. Only slightly decreased concentrations of the large, neutral amino acids were found in the chronic hyperphenylalaninaemic mice that were treated with the amino acid mixture (Fig. 4). Apparently the brain can tolerate a slight amino acid imbalance with no measurable effect on protein synthesis. But for the most part, brain protein synthesis appears to be quite vulnerable even to the 30% loss of the large, neutral amino acids observed during acute hyperphenylalaninaemia (McKean *et al.*, 1968). Most importantly, while the amino acid mixture was preventing the inhibition of brain protein synthesis resulting from chronic hyperphenylalaninaemia, the concen-

trations of phenylalanine in the brain remained greatly elevated. Because treatment of hyperphenylalaninaemic mice with the amino acid mixture had no effect on phenylalanine concentrations, but affected brain protein synthesis and the amino acid composition in the brain, it seems probable that the amino acid imbalance in the brain is the underlying mechanism of the inhibition of protein synthesis, rather than the elevated concentrations of phenylalanine itself.

Treatment of acute hyperphenylalaninaemia with the same mixture of large, neutral amino acids was reported to induce a 'recovery' of brain polyribosome profiles and elongation events (Hughes & Johnson, 1977a, 1978). The time-course analysis of the present study revealed that the mixture actually prevented the effects of increased phenylalanine on brain protein synthesis (Fig. 2). The ability of the mixture to prevent, rather than reverse, an inhibition of protein synthesis opens up the possibility that normal protein synthesis can be maintained constantly throughout the chronic hyperphenylalaninaemic condition.

We have previously shown that brain protein synthesis remains inhibited in the presence of excess phenylalanine for at least 10 days (Binek *et al.*, 1981). As few as five daily injections of α -methylphenylalanine and phenylalanine in newborn mice result in permanent learning disabilities (Luttges & Gerren, 1980). Prolonged hyperphenylalaninaemia also results in myelin deficits (Shah & Johnson, 1978; Johnson & Shah, 1980), with the primary defect involving myelin basic proteins (Figlewicz & Druse, 1980). Maintaining normal brain protein synthesis during chronic hyperphenylalaninaemia with the amino acid mixture will provide a means of determining the contribution of decreased protein-synthesis activity to the overall impairment of brain development associated with hyperphenylalaninaemia.

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