Transport and Incorporation of Labeled Compounds in Experimental Phenylketonuric Rats[1] (37667)

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Hyperphenylalaninemia induced in the rat by daily injections of phenylalanine results in various anomalies in brain chemistry. It decreases galactolipid, cholesterol (1), and nonessential amino acid (2) levels; decreases incorporation of ^{14}C-glucose into lipids (3) and ^{35}S-methionine into myelin proteins (4); and reduces the cerebral ratios of RNA to DNA and protein to DNA (5).

These biochemical effects may be caused by interference with the transport of metabolic precursors into the brain, inhibition of enzymic reactions by phenylalanine and its metabolites, or by combination of both. In the present study, the uptake by the rat brain of representative solutes was examined to see to what extent defective transport of solutes may contribute to abnormal brain biochemistry observed in experimental phenylketonuria (PKU).

Material and Methods. Timed-pregnant Sprague–Dawley rats were purchased from Simonsen's Laboratories, Gilroy, CA. At birth, the litter size in the control groups was increased (12 to 18 pups) and that in the experimental groups was reduced (6 to 8 pups), so that the difference in the growth rates in the two groups was minimized. The pups in the experimental group were injected subcutaneously with a 2% solution of phenylalanine (0.05 ml/g body wt) twice daily (2 hr apart) from Day 2 to the day of experiment. The pups in the control group were injected likewise with comparable volumes of 0.9% saline. The control dam was fed powdered lab chow while the experimental dam was fed the same diet containing 0.1% w/w of *p*-chlorophenylalanine. We reported previously that pups nursed by a dam fed this diet showed extensive inhibition of liver phenylalanine hydroxylase (6).

The uptake into brain of the solutes was studied with the internal standard method using tritiated water. A mixture of ^{14}C-labeled solute and tritiated water was injected into the tail vein, and the rats were killed 90 min thereafter. The uptake of the ^{14}C-labeled solute into the brain was calculated as follows:

$$\text{Brain uptake index} = \frac{^{14}\text{C}/^{3}\text{H in brain}}{^{14}\text{C}/^{3}\text{H in plasma}} \times 100.$$

This mode of expression is a modification of that described by Oldendorf (7) who used ^{14}C/^{3}H of the injected solution as the denominator. According to this method, the uptake of ^{14}C-labeled solute by the brain is expressed as that relative to tritiated water, the uptake of which is taken to be 100%.

Tissues were homogenized in 3 vol of water. An aliquot (0.1 ml) was pipetted into a counting vial to which was added 10 ml of Scintisol-Complete (Isolab). The samples were counted in a dual-label counting mode using a Beckman Model LS-250 liquid scintillation system.

In the glucose and glutamic acid incorporation studies, the radioactive substrates were injected subcutaneously 1.5 hr after the last saline or phenylalanine injection. Three hours later, the rats were decapitated and various fractions of the brain were isolated essentially by the methods of Agrawal, Bone and Davidson (4).

In the (^{3}H)leucine incorporation study, L-leucine-^{3}H was injected intraperitoneally 1.5 hr after the last saline or phenylalanine injection. Three hours later, the rats were decapitated, and the brain was removed. Myelin

[1] Supported by U.S. Public Health Service Research Grant HD-01972.

TABLE I. Uptake into Brain of Unmetabolizable Analogs of Glucose and Amino Acids.[a]

Group	Solute	Body wt (g)	Brain uptake index (%)
Control	α-Methyl-D-glucoside	23.7 ± 0.7	9.25 ± 0.06
Experimental		19.7 ± 1.5	10.34 ± 0.52
Control	Cycloleucine	23.7 ± 0.3	116.88 ± 8.10
Experimental		22.0 ± 1.7	57.37 ± 5.72

[a] Values are the means ± SEM. Three 12-day-old rats were used in each group. The rats were killed 90 min after subcutaneous injection of radioactive compounds. Each rat was injected with 0.01 ml/g of a solution containing 10 μCi of ^{14}C and 30 μCi of ^{3}H. Sp act: $^{3}H_2O$, 25 mCi/g; α-methyl-D-glucoside-UL-^{14}C, 2.4 μCi/μmole and 1-aminocyclopentane-1-COOH-^{14}C (cycloleucine), 54 μCi/μmole.

(8) and nonmyelin, crude preparations of Wolfgram's protein, proteolipid protein, and basic protein (9) were prepared. Aliquots (0.2 to 0.5 ml) of these fractions were counted using Scintisol-Complete.

Results and Discussion. To examine the transport phenomenon uncomplicated by metabolic transformations, the uptake by the brain of α-methyl-D-glucoside and cycloleucine was compared between the control and experimental PKU rats. The data summarized in Table I show that the uptake of the glucose analog was not altered by hyperphenylalaninemia whereas the uptake of cycloleucine was halved in the experimental group.

Although *p*-chlorophenylalanine is also present in the experimental group, the primary biochemical effect observed is believed to be ascribable to the high plasma phenylalanine level. The plasma levels of these amino acids in a typical experimental group were assayed by a gas chromatographic method (10). The average values from six rats each were 1.42 ± 0.82 (SD) mg% of phenylalanine for the control group, and 116.00 ± 47.61 mg% of phenylalanine, and 3.12 ± 1.31 mg% of *p*-chlorophenylalanine for the experimental group. These values were observed 4 hr after the second daily phenylalanine injection.

The uptake by the brain of glucose itself does not appear to be reduced by hyperphenylalaninemia. As shown in Table II, the acid-soluble supernatant fraction of the brain of both groups of rats contained approximately equal counts. It is evident then that decreased incorporation of ^{14}C-glucose into proteolipid and lipid shown here and reported by others (3, 11) is due primarily to defective utilization rather than to defective transport of glucose into the brain. Indeed, a stereospecific competitive inhibition by L-phenylalanine of brain pyruvate kinase was reported by Weber (12). Both phenylalanine and phenylpyruvate were found to increase the K_m for glucose in the *in vitro* production of lactate (13).

In contrast to glucose, the lower counts in the acid-soluble fraction after administration of ^{14}C-glutamic acid in the experimental group indicate that the uptake of this amino acid may be hindered by the presense of a high level of phenylalanine. The alternative explanation that glutamic acid may be more rapidly metabolized in the experimental group and thus less is available for brain uptake seems unlikely, since phenylalanine has been reported to inhibit the *in vitro* uptake of several other amino acids by the brain slices (14). Also, Gruemer *et al.* (15) reported that there was a general shift of most brain amino acids to a lower level after phenylalanine was injected to experimental rats for several days.

On the basis of the data obtained with cycloleucine, the uniformly decreased incorporation of ^{3}H-leucine into various protein fractions of the brain in the experimental group (Table III) may be explained by the reduced entry of leucine into the brain. Similar data for the decreased transport and incorporation of labeled methionine and leucine into myelin protein have been reported (5).

Thus, it is evident that hyperphenylalaninemia results in the inhibition of the biosynthesis of essential macromolecules in the brain by decreasing the intracellular concentration

TABLE II. Uptake of Labeled Glucose and Glutamic Acid into Various Fractions in Rat Brain.[a]

Group	Substrate	Weight (g)		cpm/g of wet brain			
		Body	Brain	Acid soluble	Proteolipid	Lipid	Protein residue
Control	Glucose	21.3 ± 0.3	0.81 ± 0.02	20,691 ± 903	1466 ± 161	9598 ± 275	13,205 ± 779
Experimental		16.3 ± 0.9	0.65 ± 0.02	19,967 ± 5621	729 ± 297	6441 ± 1230	9685 ± 1732
p		<0.001	<0.001		<0.001	<0.001	<0.005
Control	Glutamic acid	25.5 ± 0.6	0.99 ± 0.02	21,308 ± 2319	490 ± 116	2585 ± 558	7281 ± 635
Experimental		18.0 ± 1.0	0.72 ± 0.02	11,730 ± 1231	342 ± 61	2244 ± 388	4864 ± 565
p		<0.001	<0.001	<0.001	<0.05		<0.001

[a] Values are the means ± SEM. Six 9-day-old rats were used in each group. The rats were killed 3 hr after subcutaneous injection of radioactive substrates. Each rat was injected with 0.1 ml/10 g of a solution containing 10 μCi/ml of D-glucose-UL-^{14}C (sp act 5 μCi/μmole) or 10 μCi/ml of L-glutamic acid-UL-^{14}C (sp act 5 μCi/μmole).

TABLE III. Incorporation of Leucine-^{3}H into Various Brain Protein Fractions.[a]

Group	Weight (g)		Proteins (cpm/mg)					
	Body	Brain	Homogenate	Myelin	Nonmyelin	Wolfgram	Basic	Proteolipid
Control	28.4 ± 0.9	1.17 ± 0.02	1075 ± 125	869 ± 84	608 ± 73	782 ± 55	616 ± 68	567 ± 77
Experimental	28.1 ± 1.9	0.97 ± 0.04	739 ± 71	268 ± 26	297 ± 30	358 ± 41	263 ± 7	260 ± 21
p		<0.001	<0.05	<0.001	<0.005	<0.001	<0.001	<0.005

[a] Values are the means ± SEM. Seven control and eight experimental rats of 21 days old were used. The rats were killed 3 hr after intraperitoneal injection of radioactive leucine. Each rat was injected with 10 μCi/30 g of L-leucine-4,5-^{3}H; sp act 13.6 μCi/μmole.

of various amino acids and by decreasing the energy production and supply of glycolytic intermediates. The reduced transport of glucose into the brain does not appear to be a major factor in the decreased utilization of this substrate.

Summary. In hyperphenylalaninemia induced by daily injections of phenylalanine to growing rats, the entry of α-methyl-D-glucose or glucose into the brain is not hindered. Thus, defective utilization of glucose due to high levels of phenylalanine and its metabolites appears to be the reason for its reduced incorporation into lipids and proteins. On the other hand, decreased incorporation into proteins of amino acids such as glutamic acid and leucine appears to be due to the poor uptake by the brain of these amino acids.

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Received June 18, 1973. P.S.E.B.M., 1973, Vol. 144.