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Tryptophan Overload in the Pregnant Rat: Effect on Brain Amino Acid Levels and *In Vitro* Protein Synthesis

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Abstract: The concentration of most amino acids was higher in the brains of 19- and 21-day rat fetuses than in their respective mothers. After an intraperitoneal load of tryptophan to the mother, the intracerebral concentration of several amino acids (including leucine) decreased not only in the mothers, but also in their fetuses. The *in vitro* incorporation of [^{3}H]leucine into proteins in brain postmitochondrial supernatant fractions was enhanced in both the mothers and fetuses after tryptophan administration, but this effect disappeared when protein synthesis was calculated by using specific activities corrected for the amount of unlabeled leucine in the preparation. By this criterion, protein synthesis activity appeared similar in the brains of 19- and 21-day pregnant rats but was higher in their fetuses, especially in the 21-day subjects. Thus, protein synthesis in the brain was not altered by marked changes in the amino acid pool and more profound and prolonged metabolic disturbances must occur to cause permanent damage in the developing brain. **Key Words:** Tryptophan—Brain—Fetus—Pregnant rat—Protein synthesis—Amino acids. **Fando J. L. et al.** Tryptophan overload in the pregnant rat: Effect on brain amino acid levels and *in vitro* protein synthesis. *J. Neurochem.* **37**, 824–829 (1981).

The concentration of free amino acids in the brain changes during development (Oja and Piha, 1966; Levi et al., 1967; Agrawal et al., 1971; Cutler and Dudzinski, 1974). It has been shown that increased levels of different amino acids in maternal plasma produce marked alterations in the free amino acid pool of the fetal brain (Carver et al., 1965; Carver, 1969). Changes in the free amino acid pool during development may cause permanent damage in the developing brain (Fugimoto et al., 1979). Although a relationship may exist between changes in the free amino acid pool and brain protein synthesis, this has not yet been demonstrated even in mature brain. Some investigators have found that increases in the circulating concentration of certain amino acids induce brain polysome disaggregation and protein synthesis inhibition (Guroff et al., 1961; McKean et al., 1968; Aoki and Siegel, 1970; MacInnes and Schlesinger, 1971; Siegel et al., 1971; Copenhaver et al., 1973; Hughes and Johnson, 1976), while others have concluded that amino acid overload restricts the transport of other amino acids through the blood brain barrier (Pardridge and Oldendorf, 1975; Pardridge, 1977), although it stimulates the synthesis of cerebral proteins (Agrawal et al., 1970; Roberts, 1974; Antonas and Coulson, 1975).

Tryptophan is known to stimulate hepatic protein synthesis (Fleck et al., 1965; Sidransky et al., 1971), but its effect on brain protein synthesis has not yet been precisely defined (Siegel et al., 1971; Barondes, 1974). We explored the effect of tryptophan administration in pregnant rats on individual amino acid levels and brain protein synthesis in

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Abbreviations used: GABA, γ-Aminobutyric acid; PMS, Postmitochondrial supernatant; TCA, Trichloroacetic acid.

both mothers and their fetuses by using an *in vitro* free mitochondrial system (Fando and Wasterlain, 1980).

MATERIALS AND METHODS

Female Wistar rats fed a Purina Chow diet and kept in a temperature- and light-controlled room (22 ± 1°C, 12-h light-dark cycle) were mated at the age of 8–12 weeks. The day that spermatozoa were found in the vaginal smears was considered day 0 of gestation. At days 19 and 21 of gestation, half of the animals were intraperitoneally injected with 250 mg/kg body weight of L-tryptophan dissolved in 0.1 N-HCl, while the other half was injected with the medium and used as controls. Thirty minutes later all rats were killed by decapitation and the maternal and fetal brains (free of cerebellum) were rapidly dissected.

Amino Acid Determination

When used for amino acid analysis, the excised brains were immediately placed in liquid nitrogen, and weighed aliquots were homogenized in 10% sulfosalicylic acid. After centrifugation, the supernatants were brought to pH 2.2 with LiOH and analyzed in a Beckman 121 MB amino acid analyzer (Martin del Río and Latorre Caballero, 1980).

Protein Synthesis

Amino acid incorporation by brain postmitochondrial fractions was measured as described earlier (Fando and Wasterlain, 1980). Brain homogenates were made (1:4, w/v) in 50 mM-Tris HCl, pH 7.5, buffer containing 25 mM-KCl, 1 mM-dithiothreitol, and 0.35 M-RNase-free sucrose (Schwartz-Mann, Orangeburg, New York). After centrifugation for 15 min at 16,000 *g*, 0.2-ml aliquots of the postmitochondrial supernatant (PMS) were brought to a final volume of 0.3 ml containing: 0.6 mM-ATP, 0.6 mM-GTP, 12 mM-creatine phosphate, 0.02 mg creatine kinase, and 3 mM-$MgCl_2$. Then, 5.0 μCi of L-[^{3}H]leucine (specific activity 47 Ci/mmol) was added at the onset of a 15-min incubation at 37°C. The reaction was terminated by transfer to an ice bath and immediate addition of 3 ml of cold 10% trichloroacetic acid (TCA). Radioactivity incorporated into proteins was determined in the TCA precipitate as previously described (Fando and Wasterlain, 1980), and the protein concentration was determined by the method of Lowry (Lowry et al., 1951), using bovine serum albumin as standard.

Statistical analysis of the data was performed by Student's *t*-test.

RESULTS

Amino Acid Levels in the Brain of Pregnant Rats and Their Fetuses

Concentrations of free amino acids in the brains of 19- and 21-day pregnant rats and their respective fetuses are shown in Table 1. Glutamic acid was the most abundant amino acid in the mother's brain, followed by taurine and aspartic acid, and differences were minor in the amino acid profiles of these

TABLE 1. *Free amino acids in maternal and fetal brain*

	19-Day gestation		21-Day gestation	
Amino acid	Mother	Fetus	Mother	Fetus
Phosphoserine	0.25 ± 0.05	0.31 ± 0.02	0.35 ± 0.04	0.24 ± 0.03
Taurine	3.79 ± 0.49	13.45 ± 1.06^c	4.51 ± 0.18	12.92 ± 0.45^c
Phosphoethanolamine	1.52 ± 0.19	5.28 ± 0.41^c	1.73 ± 0.11	4.41 ± 0.17^c
Aspartic acid	2.35 ± 0.19	1.46 ± 0.20	2.59 ± 0.13	1.42 ± 0.26^a
Threonine	0.60 ± 0.05	1.13 ± 0.07^c	0.62 ± 0.10	0.57 ± 0.07
Serine	0.85 ± 0.05	1.27 ± 0.13^c	0.90 ± 0.01	0.84 ± 0.03
Glutamic acid	7.07 ± 0.50	4.05 ± 0.42^c	8.54 ± 0.32	3.47 ± 0.19^c
Glutamine	1.42 ± 0.26	1.30 ± 0.09	2.11 ± 0.19	1.53 ± 0.10^a
Glycine	0.72 ± 0.07	1.33 ± 0.10^c	0.79 ± 0.05	1.22 ± 0.08^c
Alanine	0.50 ± 0.04	3.37 ± 0.42^c	0.57 ± 0.03	2.83 ± 0.15^c
Valine	0.03 ± 0.01	0.28 ± 0.04^c		0.21 ± 0.02^c
Cystine	0.37 ± 0.17	0.49 ± 0.12	0.29 ± 0.04	0.24 ± 0.02
Methionine	0.02 ± 0.01	0.10 ± 0.02^c	0.03 ± 0.00	0.08 ± 0.01^b
Cystathionine	0.02 ± 0.00	0.03 ± 0.01	0.02 ± 0.00	0.07 ± 0.01^c
Isoleucine	0.02 ± 0.00	0.14 ± 0.02^c	0.03 ± 0.00	0.10 ± 0.01^c
Leucine	0.07 ± 0.01	0.39 ± 0.06^c	0.06 ± 0.00	0.27 ± 0.02^c
Tyrosine	0.02 ± 0.00	0.18 ± 0.03^c	0.04 ± 0.01	0.19 ± 0.02^c
Phenylalanine	0.03 ± 0.00	0.20 ± 0.04^c	0.03 ± 0.00	0.14 ± 0.02^b
GABA	1.68 ± 0.16	1.13 ± 0.08^c	2.12 ± 0.10	1.15 ± 0.04^c
Tryptophan	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
Ornithine	0.04 ± 0.01	0.11 ± 0.01^c	0.05 ± 0.01	0.09 ± 0.01^b
Lysine	0.23 ± 0.00	0.53 ± 0.04^c	0.25 ± 0.02	0.46 ± 0.03^c
Histidine	0.06 ± 0.01	0.14 ± 0.02^c	0.05 ± 0.01	0.15 ± 0.01^c
Arginine	0.10 ± 0.01	0.15 ± 0.02^a	0.09 ± 0.00	0.12 ± 0.01

Values are expressed as μmol/g tissue ± S.E.M. and represent the averages of 4–5 animals. Statistical comparisons between fetus and mothers: $^a p < 0.05$; $^b p < 0.01$; $^c p < 0.001$.

two groups of pregnant rats. Amino acid levels in the fetal brains were significantly higher than in their mothers, although phosphoserine, aspartic acid, glutamine, and cystine levels were unchanged or slightly reduced and the levels of glutamic acid and γ-aminobutyric acid (GABA) were always lower. The most abundant amino acid in the fetal brain was taurine. Minor differences appeared in the amino acid profiles of 19- and 21-day fetuses, although threonine, isoleucine, and leucine were significantly reduced ($p < 0.001$, <0.005, and <0.01, respectively) and cystathionine was significantly enhanced ($p < 0.001$) in the older fetuses.

Effect of Tryptophan Administration on Brain Amino Acid Levels in Pregnant Rats

As shown in Table 2, 30 min after the intraperitoneal injection of tryptophan in pregnant rats, along with an increase in tryptophan, significant reductions in leucine and phenylalanine concentrations occurred in 19-day pregnant rats, with similar reductions of glutamine, methionine, and leucine in the 21-day-pregnant subjects. Amino acid levels in the fetal brains also changed after administration of tryptophan to the pregnant animals. In rats at 19 days gestation there were significant reductions in the levels of valine, methionine, leucine, tyrosine, phenylalanine, and histidine. Tryptophan administration to the 21-day-pregnant rats raised the brain levels of phosphoserine, threonine, serine, and glutamic acid and decreased methionine, leucine, and histidine in their fetuses.

Effect of Tryptophan on *In Vitro* Brain Protein Synthesis

Brain protein synthesis was estimated by the *in vitro* incorporation of [^{3}H]leucine into proteins by postmitochondrial supernatant preparations. As shown in Table 3, that parameter remained stable in the brains of 19- and 21-day pregnant subjects. In the 19-day but not the 21-day fetuses, the net incorporation of [^{3}H]leucine into brain proteins was significantly reduced compared to their respective mothers. However, when the radioactive values were corrected for dilution of the tracer with endogenous leucine, protein synthesis increased 200 and 400% in the brains of 19- and 21-day fetuses in comparison with their mothers. Brain protein synthesis was always greater in the 21-day than in the 19-day fetuses. After tryptophan loading in the mothers, there was significantly increased incorporation of [^{3}H]leucine into brain proteins in both mothers and fetuses. However, when correcting these values with the endogenous concentration of leucine, ac-

TABLE 2. *Free amino acids in maternal and fetal brain 30 min after tryptophan administration to the mother*

Amino acid	19-Day gestation		21-Day gestation	
	Mother	Fetus	Mother	Fetus
Phosphoserine	0.32 ± 0.03	0.26 ± 0.05	0.31 ± 0.02	0.33 ± 0.01↑
Taurine	4.32 ± 0.45	14.66 ± 0.83^c	3.43 ± 0.26	13.58 ± 0.44^c
Phosphoethanolamine	1.51 ± 0.06	5.14 ± 0.72^c	1.36 ± 0.14	5.16 ± 0.46^c
Aspartic acid	2.34 ± 0.12	1.45 ± 0.12	2.26 ± 0.11	1.68 ± 0.08^b
Threonine	0.59 ± 0.05	1.07 ± 0.13^c	0.50 ± 0.03	0.79 ± 0.04^c↑
Serine	0.80 ± 0.04	0.99 ± 0.05	0.80 ± 0.04	1.05 ± 0.07^a↑
Glutamic acid	6.91 ± 0.29	3.67 ± 0.13^b	7.26 ± 0.37	4.39 ± 0.18^c↑
Glutamine	1.17 ± 0.14	1.21 ± 0.27	1.45 ± 0.06↓	1.43 ± 0.30
Glycine	0.72 ± 0.03	1.16 ± 0.12^c	0.87 ± 0.05	1.33 ± 0.06^c
Alanine	0.55 ± 0.02	2.56 ± 0.13^c	0.61 ± 0.03	2.70 ± 0.19^c
Valine	0.03 ± 0.00	0.16 ± 0.02^c↓	0.06 ± 0.01	0.19 ± 0.02^c
Cystine	0.46 ± 0.14	0.31 ± 0.07	0.41 ± 0.03	0.34 ± 0.05
Methionine	0.01 ± 0.00	0.05 ± 0.01^a↓	0.02 ± 0.00	0.05 ± 0.01^b↓
Cystathionine	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.07 ± 0.00^c
Isoleucine	0.02 ± 0.00	0.10 ± 0.01^c	0.02 ± 0.00	0.07 ± 0.01^c
Leucine	0.04 ± 0.00↓↓	0.24 ± 0.03^c↓	0.04 ± 0.01↓	0.19 ± 0.02^c↓
Tyrosine	0.02 ± 0.00	0.07 ± 0.00^a↓↓	0.04 ± 0.02	0.13 ± 0.02^a
Phenylalanine	0.02 ± 0.00↓	0.11 ± 0.01^b↓	0.03 ± 0.01	0.11 ± 0.02^a
GABA	2.01 ± 0.05	0.93 ± 0.08^c	2.17 ± 0.09	1.14 ± 0.05^c
Tryptophan	0.12 ± 0.02↑↑↑	0.36 ± 0.08↑↑↑	0.30 ± 0.18↑↑↑	0.23 ± 0.07↑↑↑
Ornithine	0.05 ± 0.01	0.10 ± 0.01^b	0.05 ± 0.01	0.09 ± 0.01^b
Lysine	0.28 ± 0.05	0.44 ± 0.05^b	0.27 ± 0.02	0.53 ± 0.03^c
Histidine	0.05 ± 0.01	0.08 ± 0.01↓	0.04 ± 0.01	0.10 ± 0.01^b↓
Arginine	0.09 ± 0.02	0.13 ± 0.01	0.09 ± 0.01	0.13 ± 0.01^b

Values are expressed as μmol/g tissue ± S.E.M. and represent the averages of 4–5 animals.

Statistical comparisons between fetus and mother are shown as follows: $^a p < 0.05$, $^b p < 0.01$, $^c p < 0.001$; those between tryptophan and control groups by arrows: ↑ or ↓, $p < 0.05$; ↑↑ or ↓↓, $p < 0.01$; ↑↑↑ or ↓↓↓, $p < 0.001$.

TABLE 3. *[^{3}H]Leucine incorporation into proteins by postmitochondrial supernatants from maternal and fetal brain: effect of tryptophan administration to the mother*

Group	19-Day gestation			21-Day gestation		
	[^{3}H]Leu (cpm/mg protein)	Leu in PMS (nmol/ml PMS)	Leu (pmol/mg protein)	[^{3}H]Leu (cpm/mg protein)	Leu in PMS (nmol/ml PMS)	Leu (pmol/mg protein)
Control						
Mother	19,058 ± 1426	18.6 ± 0.94	36 ± 3	24,496 ± 2469	17.0 ± 1.0	46 ± 5
Fetus	12,538 ± 934	54.2 ± 6.83	71 ± 7	23,086 ± 3459[a]	74.1 ± 3.94	182 ± 27[b]
p	<0.01	<0.01	<0.01	n.s.	<0.001	<0.001
Tryptophan						
Mother	27,761 ± 1400↑↑	13.5 ± 1.49	37 ± 6	32,660 ± 1313[a]↑↑	12.6 ± 1.51	41 ± 3
Fetus	17,830 ± 1708↑	41.5 ± 7.30	83 ± 15	33,041 ± 2129[c]↑	44.0 ± 4.40	148 ± 12
p	<0.001	<0.01	<0.05	n.s.	<0.01	<0.001

Values are expressed as means ± S.E.M. of 5–9 animals per group.

The statistical comparisons between mother and fetus are shown by the *p* values, while between 21- and 19-day gestation they are as follows: [a]$p < 0.05$, [b]$p < 0.01$; [c]$p < 0.001$; and that between tryptophan and controls by arrows: ↑, $p < 0.05$, ↑↑, $p < 0.01$. n.s., not significant.

The estimated values of leucine incorporated (pmol) were calculated correcting the incorporation data on the basis of free leucine concentration in PMS as shown in the table.

tual protein synthesis did not appear altered in either preparation by the tryptophan treatment.

DISCUSSION

Levels of free amino acids in fetal rat brains found in the present study are similar to those reported by other investigators (Carver et al., 1965; Carver, 1969). Our fetal subjects contained more free amino acids in the brain than did their respective mothers, with the exception of a decrease in those amino acids having a direct energetic and/or neurotransmitter role such as glutamic acid, glutamine, and GABA. These relative values may be compared with those previously reported in fetal and maternal plasma (Arola et al., 1977; Palou et al., 1977), and they suggest that amino acid levels in the brain depend not solely on their concentration-dependent active uptake through the blood-brain barrier, but also on their endogenous synthesis (in the case of nonessential amino acids) and specific subsequent utilization by the brain. This must be higher for energy-yielding and/or neurotransmitter amino acids than for others. The high taurine levels observed here in fetal brains correspond to those reported in immature brains (Tallan, 1962; Gaitonde, 1970; Agrawal et al., 1971), which rapidly decrease during postnatal life (Oja and Piha, 1966; Cutler and Dudzinski, 1974).

Injection of tryptophan into pregnant rats altered the composition of the free brain amino acid pool of mothers as well as of fetuses. It was also observed that after tryptophan administration to the mothers, except for a significant increment in tryptophan plasma levels, there were no other amino acid changes in either the maternal or fetal plasma (data not shown). Thus, the changes in brain amino acid levels must be due to competitive inhibition between tryptophan and certain amino acids coming into the brain and not to changes in their circulating levels. Similar conclusions have been reported when mothers were treated with other amino acids (Carver et al., 1965; Carver, 1969).

Although an increased circulating level of certain amino acids may (Guroff et al., 1961; McKean et al., 1968; Aoki and Siegel, 1970; MacInnes and Schlesinger, 1971; Siegel et al., 1971; Copenhaver et al., 1973; Hughes and Johnson, 1976) or may not (Agrawal et al., 1970; Roberts, 1974; Antonas and Coulson, 1975) interfere with protein synthesis in the brain, our results demonstrate that tryptophan overload to the mother does not affect the rate of *in vitro* leucine incorporation into the brain postmitochondrial fractions of either the mother or fetus. Although the methodology used here has been validated as a precise index of brain protein synthesis *in vitro* (Fando and Wasterlain, 1980), any extrapolation to the *in vivo* situation should be interpreted with caution. In any case, the method was sensitive enough to detect a marked rise in protein synthesis between the brains of all fetuses and their mothers and between the fetuses of 19 and 21 days. These results are in agreement with those of Gilbert and Johnson (1974) in the mouse and run in parallel with the rapid fetal brain growth during the late gestation period in the rat (Salinas and Galan, 1979).

It has been demonstrated that protein synthesis in brain and liver is dependent on the presence of amino acids in adequate amounts (Hanking and Roberts, 1965; Roberts and Morelos, 1965). Our data suggest that the rate of brain protein synthesis in PMS is not directly dependent on specific levels of amino acids, provided that minimal amounts of substrate are available. This hypothesis is in agreement with the work of Hughes and Johnson (1977) showing that increased levels of phenylalanine pro-

duce a significant decrease in certain brain amino acid concentrations, while aminoacyl-tRNA for those same amino acids are slightly increased. These considerations are compatible with the combined presence of both high levels of amino acid and increased brain protein synthesis as seen in this study for the rat fetus at late gestation. Differences between our findings and those of other investigators may be due not only to the different experimental approach, but to our use of specific activities to correct for the actual amount of unlabeled substrate incorporated into the proteins.

The lack of effect of tryptophan overload on protein synthesis in the fetal brain does not diminish the importance of prolonged changes in the free amino acid pool during development of the CNS. The present study indicates that marked alterations in the brain level of amino acids do not interfere with protein synthesis, and more profound and permanent metabolic disturbances in the mother must occur to produce brain damage in the developing fetus.

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