DEVELOPMENTAL CHANGES IN AMINOACYLATION OF MOUSE BRAIN tRNA

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SUMMARY: The concentration of tRNA for seven amino acids and the percentage of each tRNA that is aminoacylated in vivo were determined at various stages of postnatal brain development. When normalized on a brain weight basis, the amount of amino acid acceptance of $tRNA^{Phe}$, $tRNA^{Val}$ and $tRNA^{Glu}$ in 1-day old brain tissue was 50% to 100% higher than that measured in the adult brain. However, the percentage of tRNA molecules which is aminoacylated in vivo was shown to gradually decrease during neural maturation. Age-dependent changes in the proportions of aminoacylated-tRNA were shown to be independent of the concentrations of free amino acids. This phenomenon is discussed with regard to free amino acid pools and the availibility of these precursors to protein synthesis during various stages of neural development.

It has been previously suggested that variations in the intracellular concentrations of amino acids influence the rate of protein synthesis in both bacterial and mammalian cells (1,2,3). However, the dynamics between free amino acid pools and their participation in protein synthesis in the eukaryotic cell is presently far from clear. Protein synthesis in mammalian brain tissue has been shown to undergo a dramatic quantitative and qualitative alteration during early postnatal development (4,5) although the concentrations of most amino acids do not appear to significantly change (6). Recent studies regarding macromolecular metabolism in both liver and brain cells have shown that all intracellular amino acids do not participate in polypeptide synthesis to the same extent (7,8). However, the reasons for this selectivity are presently not known. In order to obtain a more meaningful assessment of the availability of amino acids for translational events, the concentrations of the immediate precursor to protein synthesis, aminoacyl-tRNA, were measured at various stages of neural development. These

studies allowed us to correlate the in vivo concentrations of amino acids and aminoacylated-tRNA during this critical period of brain maturation.

MATERIALS AND METHODS: Aminoacyl-tRNA was isolated at an acidic pH by a modification of the technique described by Allen et. al. (9). In order to minimize acylation or deacylation of tRNA during the isolation procedure, whole brains of Swiss albino mice were quickly removed and immediately placed into a Waring blendor which contained 75 ml of Na-acetate buffer (150 mM NaCl, 50 mM Na-acetate; pH 5.1), 75 ml of distilled phenol which was presaturated with Na-acetate buffer and 120 mg of bentonite. All procedures were carried out at 2-4°. After the addition of each brain, the tissue was homogenized and the final homogenate was centrifuged to separate the phenol and aqueous phases. The resulting aqueous phase was deproteinized further by two additional extractions with phenol and finally chloroform plus 1% isoamyl-alcohol. Nucleic acid was precipitated with two volumes of ethanol at -20°. Precipitates were resuspended in the Na-acetate buffer solution and ribosomal RNA removed by the addition of NaCl to a final concentration of 1.0 M. The concentration of tRNA in the final preparation was determined spectrophotometrically at 260 mu. The samples were then divided into two equal fractions, one of which was treated with a 100 times molar excess of $NaIO_4$ to oxidize nonacylated-tRNA (9) while the other, as a measure of total acceptor activity, received water. The samples were maintained for 15 minutes at room temperature in the dark after which the RNA was precipitated with ethanol and dialyzed overnight against 2000 volumes of water at 4°. Both samples of the brain tRNA were then deacylated by incubation for 3 hours at 37° in 0.2 M Tris-HCl buffer (pH 8.8).

Aminoacyl-tRNA synthetases were prepared from a post-microsomal fraction of brain (10). The fraction was made 75% saturated with $(NH_4)_2SO_4$ at 4° and the precipitate was dissolved in a buffer solution containing 10 mM Tris-HCl, pH 7.8; 10 mM $MgCl_2$ and 6 mM 2-mercaptoethanol. The resulting solution was filtered through a Sephadex G-50 column (10) and fractions containing protein

were pooled, concentrated by $(NH_4)_2SO_4$ precipitation and dialyzed against the buffer at 4^0 overnight. Protein concentrations were determined by the method of Lowry et. al. (11).

RESULTS AND DISCUSSION: The choice of amino acids in the measurement of in vivo aminoacylated-tRNA in developing mouse brain tissue was based on: a) the chemical nature of the precursors; alanine and valine are neutral aliphatic molecules, lysine is basic, tryptophan and phenylalanine are neutral aromatic compounds and aspartic acid and glutamic acid are dicarboxylic amino acids. and b) the changes in free amino acid concentration during postnatal mouse brain development; alanine, phenylalanine and valine decrease, aspartic acid and glutamic acid significantly increase while lysine remains constant (6).

Table 1 includes a comparison of total acceptor activity of tRNA, with regard to the seven amino acids listed above, that was measured with 1-day old and adult (over 9 months old) mouse brain tissue. The amount of $tRNA^{Ala}$, $tRNA^{Try}$, $tRNA^{Asp}$ and $tRNA^{Lys}$ slightly decrease between birth and maturity. The concentrations of $tRNA^{Phe}$ and $tRNA^{Val}$ in the 1-day brain is approximately 50% greater. However, the greatest difference between neonatal and mature brain tissue tRNA was found with $tRNA^{Glu}$ where the younger tissue had approximately twice the total acceptor activity. The changes in these tRNA acceptor activities probably represent a reasonable sampling since, on a molar basis, the total tRNA concentration decreases by approximately 30% from birth to maturity (Chou and Johnson, unpublished observations). The difference in in vivo aminoacylation of tRNA in 1-day old and adult mouse brain tissue was quite striking. The proportion of tRNA that is aminoacylated in the younger animals is significantly higher. Even though glutamic acid is present in significantly higher concentrations in the adult brain tissue, the level of aminoacylated-$tRNA^{Glu}$ is only 16% of that in the immature brain. In addition, although the lysine concentration does not appreciably change during neural maturation, aminoacylated $tRNA^{Lys}$ was only 50% of that measured in the 1-day old tissue. It is readily apparent that there was no simple relationship be-

Table 1

Total tRNA acceptor activity and *in vivo* aminoacylated-tRNA in 1-day old and adult mouse brain tissue.

	Total tRNA acceptor activity			In vivo aminoacylated-tRNA		
tRNA	1-day	Adult	1-day/Adult	1-day	Adult	1-day/Adult
Ala	345±12	279±4	1.2	276± 7	172±9	1.6
Try	52± 6	44±2	1.2	35± 3	20±3	1.8
Asp	114±10	99±6	1.2	108± 2	47±2	2.3
Lys	129±14	99±7	1.3	125± 8	55±2	2.3
Phe	120± 2	82±3	1.5	99± 5	53±3	1.9
Val	135± 8	93±2	1.5	132±10	44±4	3.0
Glu	123± 9	58±5	2.1	117± 8	20±2	5.9

Aminoacylation of tRNA was carried out in a reaction mixture (0.5 ml) containing 20 mM Tris-HCl, pH 7.8; 14.3 mM 2-mecaptoethanol; 0.1 mM CTP, 6 uM of 19 nonradioactive amino acids; 0.4 to 1.2 A_{260} units of tRNA; 0.18 to 0.32 mg of synthetase protein; $MgCl_2$ at optimum concentrations for each reaction as follows: 1.25 mM (tryptophan and glutamic acid), 2.5 mM (aspartic acid) and 10 mM (alanine, lysine, phenylalanine and valine); 0.75 mM ATP (alanine, tryptophan, aspartic acid, lysine and valine) or 1.5 mM ATP (phenylalanine and glutamic acid); and 1 to 2 uCi of ^{14}C-labelled amino acid (^{3}H in the case of tryptophan). The reactions were incubated at 37° for 20 minutes and terminated by the addition of 0.5 ml of cold 10% trichloroacetic acid and 0.5 mg of bovine serum albumin as a carrier. The precipitates were collected, washed twice with cold 5% trichloroacetic acid and dissolved in 0.5 ml of alkaline water. Suitable aliquots were counted in a Beckman LS-100 liquid scintillation system (10). The results are expressed as the pmoles of tRNA per gram of brain wet weight (± S.D.) for at least 3 independent preparations of tRNA.

tween the extent of aminoacylated-tRNA and the concentrations of specific amino acids in mouse brain tissue. Several types of controls were tested in order to evaluate the above data concerning *in vivo* aminoacylation of tRNA. When all tRNA molecules were deacylated prior to periodate oxidation, no acceptor activity remained. In addition, *in vitro* aminoacylation of "stripped"

tRNA completely protected the complexes against oxidation with periodate. The possibility that periodate-oxidized tRNA was inhibitory to the enzymatic aminoacylation was also examined. No inhibitory effect was observed when excess amounts of oxidized tRNA were added to the reaction mixtures. The experimental procedure probably reflected an accurate representation of the in vivo aminoacylation levels since a significant amount of deacylation during the isolation would not have resulted in the high and consistent values for neonatal brain tissue. Aminoacylation during the isolation was unlikely since the immediate homogenization of the tissue in cold phenol would eliminate all enzymatic activity. The lack of parallelism between the total tRNA acceptor activity, the in vivo levels of aminoacylation and the concentration of free amino acids suggests that the availability of amino acids for protein synthesis is restricted. In part, especially in the case of glutamic acid, simple correlations may be complicated by intracellular compartmentalization and metabolism other than polypeptide synthesis (12).

In order to assess the possible regulatory role of the level of aminoacyl-tRNA on the rate of protein synthesis during early development, tRNA was examined in brain tissue of several aged animals. Although the rate of protein synthesis rapidly declines during early neural maturation, decreasing by approximately 70% by the time the animals are 9-days of age (4), it is clear from Table 2 that the proportion of aminoacylated-tRNA is little affected. In general, the levels of aminoacylated-tRNA in vivo decrease gradually after birth and reach approximately 50% of the original levels when the animals are mature. This suggests that the loss in translational events during early neural development is not the result of an inavailability of amino acids or the levels of tRNA aminoacylation. These studies show that neither the total tRNA acceptor activity nor the in vivo acylated levels directly correlate with the free amino acid content of brain tissue. This suggests that one must be quite cautious in extrapolating rates of protein synthesis by simple arithmetical corrections with free amino acid pool sizes.

Table 2

In vivo levels of aminoacylated-tRNA
during postnatal development of mouse brain.

tRNA	Age of mice				
	1-day	9-day	34-day *	70-day *	Adult
			(%)		
Ala	82±5	92±5	67	77	59±5
Try	74±4	71±5	73	65	45±6
Asp	96±4	88±3	87	70	48±3
Lys	98±3	81±2	71	65	55±2
Phe	85±4	78±3	65	68	64±2
Val	97±3	61±4	-	-	47±2
Glu	95±5	96±5	86	75	38±7

The results are expressed as the percent of the total tRNA that was aminoacylated *in vivo* (±S.D.) by three independent preparations of tRNA (*) one tRNA preparation.

Although more laborious, the measurement of aminoacyl-tRNA, the direct precursor to protein synthesis, may provide a more meaningful measurement of amino acid availability for translational events.

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