Precursor and age-dependent differences in *in vivo* rates of protein synthesis in the cerebral cortex

Although numerous reports have appeared dealing with the effect of age on cerebral protein synthesis *in vitro*[4–10,16,18], it is not yet firmly established that these studies faithfully reflect the *in situ* relationships which tune the synthetic performance of brain tissue to its maturational development. In 1966, Johnson and Luttges[5] reported a rapid loss with increasing age of the ability of mouse brain cells to incorporate amino acids into protein *in vitro*. Further studies with ribosomal preparations[4] confirmed this finding and revealed that, although the poly-U directed synthesis decreased with age[6], no age-dependent differences in the binding of poly-U to template RNA could account for this decrease[7]. Changes in the aggregation state[17] and in the stability[18] of polysomes have also been reported during neural development, yet a recent study failed to note significant age-dependent modifications of the ability of brain polysomes to incorporate [^{3}H]leucine[18]. Roberts *et al.*[11] summarized the conflicting information by stating that the 'overall capacity of the brain for protein and nucleic acid synthesis varies little with age'.

Our past efforts in the field of cerebral protein synthesis have shown that free and endoplasmic reticulum-bound polysomes of the immature brain cortex actively participate in this process[13] and that qualitatively different nascent polypeptides are among those formed on each of the two polysomal populations[14]. Most recently we also reported that in the developing brain cortex the rates of incorporation of [^{14}C]-phenylalanine *in vivo* into the nascent polypeptides of nerve and glial cells[3,12] are quantitatively different. In the present study we compare the formation *in vivo* of nascent polypeptides on the free and the endoplasmic reticulum-bound polysomes of the 7- and 18-day-old brain cortex 10 min after the intracerebral administration of two different precursors, [^{14}C]leucine and [^{14}C]phenylalanine. In order to assess fully the effects of the interplay between the type of precursor and the age of the animal on the rates of protein synthesis, we determined at each age (a) the endogenous levels of the precursors in the cortex; (b) the TCA-soluble radioactivity and the extent of the conversion of the radioactive precursors to other radioactive, TCA-soluble metabolites; (c) the specific radioactivity of the nascent proteins; (d) the pool-corrected specific radioactivity of the nascent proteins; and (e) the molar incorporation of the precursors into the proteins taken to represent the most faithful index of the actual rate of protein synthesis. We chose 7- and 18-day-old animals because at 7 days the cortex contains few if any mature glial cells, fully grown dendritic processes and synaptic contacts, while at 18 days it has abundant glial cells and a virtually complete synaptic organization and, moreover, myelination is in its final phase.

The results of the determinations are shown in Table I. It may be seen (line 1) that the cortical levels of leucine and phenylalanine changed very little between days 7 and 18 and that the levels of leucine exceeded those of phenylalanine by about 50% at both ages[1]. Table I also shows (line 2) that 300% more [^{14}C]leucine and 500% more [^{14}C]phenylalanine entered the TCA-soluble pool of the 7-day-old cortex than that of the 18-day-old cortex. Additionally, thin-layer chromatography (*n*-butanol–glacial

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TABLE I

NASCENT CORTICAL PROTEINS SYNTHESIZED BY 7- AND 18-DAY-OLD RATS AFTER A SINGLE INTRACEREBRAL INJECTION OF [^{14}C]LEUCINE AND [^{14}C]PHENYLALANINE*

The free polysomes (FP) were isolated as described by Sellinger *et al.*[14]. The band of material containing the polysomes attached to the endoplasmic reticulum and which was recovered from the 0.5 *M*–2.0 *M* sucrose interface[14] was frozen overnight at —20°C. After thawing,the suspension was treated with Na-deoxycholate (pH 7.0; 0.3% (w/v), final concentration) for 10 min at 0°C and was centrifuged at 204,000 × *g* for 60 min. The resulting pellet represents fraction BP.

To obtain the [^{12}C]amino acid values shown on line 1, the cerebral cortex of a single rat was dissected immediately after death by decapitation and was homogenized (10 strokes at about 1,500 rev./min) in 10% (w/v) ice-cold TCA (3 ml/g of fresh weight). The suspension was centrifuged for 20 min at 15,000 rev./min; the pellet was resuspended, washed with 5 ml of 10% TCA and the suspension centrifuged as above. The washing process was repeated once more. TCA was removed by repeated (3 ×) extraction of the pooled supernatants (about 15 ml) with an equal volume of ether. The aqueous phase was filtered through 0.45 μm pore-size Metricel filters and the filtrate was lyophilized. The dry residue was suspended in 1 ml of 0.067 *M* citrate buffer, pH 2.2, and the amino acids were quantitated by automated analysis using the Beckman amino acid analyzer (model 120C). To obtain the specific radioactivities listed on lines 2 and 3, rats were lightly anesthetized with ether and were injected intracerebrally with less than 50 μl of precursor. Each 7-day-old rat received 2.5 μCi and each 18-day-old rat received 6.25 μCi in a single injection applied manually at the level of the midline. Ten minutes later the fully awake rats were decapitated, the cortices were dissected out as free as possible of subjacent white matter and, after pooling of tissue from two animals, the cortices were homogenized manually (10 up-and-down strokes) in 0.02 *M* Tris buffer, pH 7.2, containing 0.005 *M* Mg acetate and 0.025 *M* KCl[13,14]. The total radioactivity of the homogenate was determined by counting aliquots in a vial containing up to 1 ml of water, 2 ml of Biosolv-3 (Beckman Co., Fullerton, Calif.) and 10 ml of toluene scintillant[14]. To determine the TCA-insoluble radioactivity (line 3), 5 ml of ice-cold 10% TCA were added to another aliquot of the homogenate. After 15 min on ice, the suspension was heated for 30 min at 90°C and was then allowed to cool on ice for another 30 min. The TCA-soluble radioactivity (line 2) was determined by subtraction of the TCA-insoluble radioactivity from the total radioactivity. All measurements of radioactivity were performed in a Nuclear Chicago (Unilux II) spectrometer. The counting efficiency ranged between 80 and 85%. The counts/min were converted to disint./min using a suitable program on the PDP-8 computer.

The values on line 4 refer to the pool-corrected specific radioactivities obtained, in a given experiment, by dividing the values of the TCA-insoluble specific radioactivity by the TCA-soluble specific radioactivity. The means of the individual ratios thus generated are shown. The molar incorporations (line 5) were obtained by dividing the TCA-insoluble specific radioactivity values by the specific radioactivity of the precursor amino acid; the latter was calculated in each experiment from the C^{12}-values listed on line 1 and on the valid assumption that the TCA-soluble radioactivity (line 2) was mostly, if not totally, unchanged precursor. In all the calculations, the values of 61.3 and 113 mg of protein/g of cortex were used for the 7- and 18-day-old animals, respectively. The ratios shown are of the means of the individual experiments. H = homogenate; FP = free polysomes; BP = bound polysomes; s.r.a. = specific radioactivity. Between parentheses is the number of determinations.

Variable determined (units)	*[^{14}C]Leucine*					
	7-day			*18-day*		
	H	*FP*	*BP*	*H*	*FP*	*BP*

Variable determined (units)	*[^{14}C]Phenylalanine*					
	7-day			*18-day*		
	H	*FP*	*BP*	*H*	*FP*	*BP*
1. [^{12}C]Amino acid (μmoles/g)	0.038 ± 0.001 (3)	—	—	0.044 ± 0.008 (8)	—	—
2. TCA-soluble s.r.a. (disint./min/mg of protein)	28,755 ± 3,437 (4)	—	—	5,219 ± 810 (10)	—	—
3. TCA-insoluble s.r.a. (disint./min/mg of protein)	9,304 ± 1,563 (4)	24,301 ± 209 (2)	22,416 ± 1,712 (2)	2,635 ± 415 (10)	8,027 ± 1,876 (2)	6,172 ± 1,026 (2)
4. Pool-corrected s.r.a. (3./2.)	0.32 ± 0.03	0.85 ± 0.00**	0.78 ± 0.07**	0.55 ± 0.05	1.60 ± 0.16**	1.26 ± 0.22**
5. Molar incorporation (pmoles/mg of protein)	211 ± 41	550 ± 1	508 ± 45	204 ± 36	631 ± 6	497 ± 82

* Where 3 or more determinations were made, means ± S.E.M. are given; where 2 determinations were made, the average ± range from the average value is given.

** Obtained by dividing s.r.a. of line 3 by the TCA-soluble s.r.a. of the corresponding homogenate (line 2).

acetic acid–water, 4 : 1 : 5, v/v) revealed that more than 75% of the TCA-soluble radioactivity was unchanged [^{14}C]leucine and more than 95% was unchanged [^{14}C]-phenylalanine. The incorporation of the [^{14}C]amino acids into TCA-insoluble form is shown on line 3 of Table I, while the pool-corrected TCA-insoluble specific radioactivity (PCSA) appears on line 4. Examination of the PCSA values reveals (a) higher specific radioactivity values for the proteins of the 18- than for those of the 7-day-old cortex at all levels of comparison (homogenate (H), free polysomes (FP) and membrane-bound polysomes (BP)) and with both precursors and (b) higher incorporations into the free than into the membrane-bound polysome-associated nascent polypeptides, with a greater FP–BP difference for leucine than for phenylalanine. Finally, a comparison of the molar incorporations of leucine and phenylalanine into the cortical proteins (line 5), which provides the best quantitative assessment of the actual rates of protein synthesis, reveals (a) that the highest rate of incorporation was that of leucine into the nascent polypeptides associated with the free polysomes of the 18-day-old cortex and (b) that synthesis of the cerebral proteins is largely precursor- and age-dependent, since the molar incorporation of leucine increased markedly between days 7 and 18 while, conversely, that of phenylalanine remained constant over the same time period.

We may compare the results of Table I to the previous findings of Oja[10] who showed identical molar incorporations of [^{3}H]tyrosine into the brain proteins of 7- and 14-day-old rats *in vivo*, of Szijan *et al.*[15] who reported decreasing PCSA values with age for the [^{14}C]phenylalanine pulse-labelled cortical, cerebellar and hypothalamic, but not for the hypophyseal proteins, and of Dainat *et al.*[2] who observed higher PCSA values for cerebellar [^{14}C]leucine pulse-labelled proteins at 14 than at 7 days post-natally. Finally, it is worth mentioning that Zomzely *et al.*[18] noted an age-dependent difference in the partition of [^{3}H]leucine pulse-labelled nascent proteins between the light and the heavy ribosomal aggregates and that in similar experiments in which preparations of polysomes were examined in linear sucrose density gradients, we observed a larger percentage of the [^{14}C]leucine pulse-labelled nascent proteins to associate with the monomer + dimer + trimer region in the cortices of 18-day-old animals than in those of 7-day old animals.

We believe that the results shown in Table I should help reconcile some of the conflicting literature findings listed above, inasmuch as they directly document the fact that brain cells utilize amino acids unequally during their maturation. We therefore recommend that future measurements of cerebral protein synthesis during development be carried out with this constraint in mind.

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