PROTEIN TURNOVER DURING MATURATION OF MOUSE BRAIN TISSUE

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ABSTRACT

The measurement of protein turnover involves the product of the rates of protein synthesis and degradation. It is the dynamic balance between these two components that determines the measured net rate of protein synthesis. The data reported here show that brain cells from newborn animals incorporate arginine-^{14}C into acid-insoluble protein at a rate 10-fold greater than the rate for brain cells obtained from 15-day old animals. This difference in incorporation occurred even though the rate of arginine accumulation and the resulting pool size of radioactive precursor were similar for both ages. The measurement of protein turnover in brain cell suspensions prepared from 1-day old animals was shown to be complex and to exhibit a cyclic phenomenon in regard to arginine-^{14}C incorporation into and release from protein. The variation in half-life calculations (0.5–3.5 hr) due to this cyclic phenomenon is discussed. Although puromycin was added in an attempt to amplify the rate of degradation by preventing the synthesis of new protein, it was found that degradation was inhibited as well, suggesting a relationship between protein synthesis and degradation.

INTRODUCTION

Several previous studies of protein turnover in neural tissue have been made. In general, it has been shown that protein synthesis appears to be more intense during brain growth (3, 7) and nerve cell regeneration (11, 12). However, accurate measurements of turnover rates have been exceedingly complex since many studies have utilized in vivo techniques which are complicated by the presence of the blood-brain barrier, catabolism of newly synthesized proteins, and the reutilization of the labeled amino acids as well as the heterogeneity of the proteins and cell types (13).

It has been shown previously that the apparent rate of protein synthesis in mouse brain cell suspensions rapidly decreases during early postnatal development (7). By the time the animals were 12 days of age, the rate of amino acid incorporation into acid-insoluble protein decrease by 90% and approached levels measured in adult brain tissue. Since cellular protein metabolism is a dynamic system which is characterized by an equilibrium between active synthesis and degradation, it is the balance between these rates that determines the measured net gain or loss of cellular protein. The purpose of this study was to analyze the relationship between amino acid incorporation into cellular protein and the rate of protein degradation during early postnatal brain development.

MATERIALS AND METHODS

Brain cell suspensions were prepared in Eagle's minimal essential medium, supplemented with 5% dialysed calf serum, and incubated with arginine-^{14}C or valine-^{14}C (0.5 μCi/ml) under an oxygen atmosphere as previously described (7). The amount of radioactivity incorporated into protein was measured by precipitation with hot trichloroacetic acid (TCA),

while the amount of free, intracellular amino acid (TCA soluble) was determined by taking a 0.2 ml sample of the supernatant from the first TCA precipitation (7).

In order to study the turnover of proteins synthesized during the incubation period, cell suspensions were incubated with either labeled arginine or valine as described above and, at selected periods, a 1-3000-fold excess of unlabeled arginine (2.5×10^{-2} M) or valine (2.0×10^{-2} M), respectively, was added directly to each tube. After flushing the culture tubes with oxygen, each reaction was allowed to continue to incubate and the reaction was stopped at selected intervals. The amount of radioactive amino acid remaining in the acid-insoluble protein and in the TCA-soluble fraction was determined as stated above.

To study the degradation process without the complication of reutilization of labeled amino acid into newly synthesized protein, puromycin was used to inhibit translational events. In these experiments, 0.2 ml of puromycin (5×10^{-4} M) was added to each tube at the end of the labeling period. After the cells were reoxygenated, incubation was continued and the radioactivity and total cellular protein were determined at appropriate intervals.

The final protein concentration of each reaction (0.25–0.50 mg/ml) was determined by the method of Lowry et al. (9). In each experiment, the results were expressed as specific activity, cpm/mg protein.

Chemicals were purchased from the following: U-^{14}C-L-arginine, (specific activity, 125 mCi/mmole) and U-^{14}C-L-valine (specific activity, 248 mCi/mmole), New England Nuclear Corp., Boston, Mass. L-arginine and L-valine, Nutritional Biochemicals Corporation, Cleveland, Ohio; and puromycin-HCl, Sigma Chemical Co., St. Louis, Mo.

RESULTS

To study the incorporation of arginine-^{14}C into TCA-insoluble protein, 1- and 15-day cell suspensions were incubated with labeled arginine for up to 60 min. In both cases, the rate of arginine-^{14}C incorporation into protein was linear for 60 min, although the rate of synthesis was eight to twelve times greater with brain cell preparations obtained from newborn animals (Fig. 1). Even though there was a 10-fold difference in protein synthesis, we found, on the average, little difference in the rate of accumulation or in the pool size of radioactive precursor between the 1- and 15-day preparations (Fig. 1).

Since labeled amino acids were more actively incorporated into protein by relatively young animals, the rate of protein turnover was initially measured with cell suspensions obtained from mouse brain tissue from animals 1 day after birth.

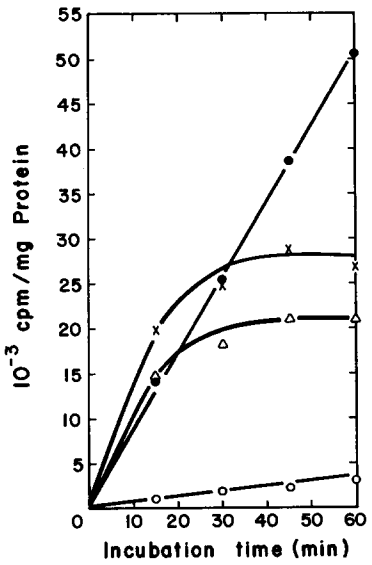

FIGURE 1 Incorporation of arginine-^{14}C into hot TCA-soluble and -insoluble material of 1- and 15-day mouse brain cell suspensions as a function of time. TCA-insoluble material: ●, 1-day; ○, 15-day. TCA-soluble material: ×, 1-day; △, 15-day.

The cells were incubated with arginine-^{14}C for increasing intervals of time. At the periods indicated (Fig. 2), an excess of unlabeled arginine was added to each tube. After a total incubation of 60 min, the amount of labeled arginine associated with acid-insoluble protein was determined. In the initial experiments, it appeared that in the presence of excess arginine in the incubation medium, degradation did occur at each of the time intervals tested (Fig. 2). However, the rate of protein turnover calculated from such studies seemed to be variable in that the amount of arginine-^{14}C lost per unit time was greater with the longer labeling and shorter chase periods. This variation could be partially, but not entirely, explained by experimental error at those intervals where there was a low amount of radiactive amino acid incorporated into protein.

This phenomenon was not unique to arginine, since similar results were obtained when cells were incubated with valine-^{14}C. Protein turnover could be monitored with both amino acids, although in repeated experiments there was considerable variation in the calculated rates for any specific interval (Table I). It was these inconsistencies in rates of degradation which suggested that protein turnover may have been quite variable and that the kinetics of protein metabolism were complex.

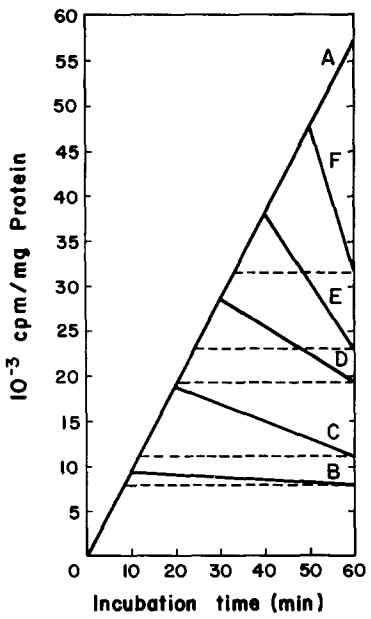

FIGURE 2 Arginine-^{14}C loss from hot TCA-insoluble material of 1-day mouse brain cell suspension as a function of time of incorporation. Cell suspensions were pulsed for 0–60 min and then chased for a total time of 60 min as described in Materials and Methods. The rates of degradation were calculated from the loss of radioactivity from the acid-insoluble protein during the reincubation period (% per min). *B*, 0.4; *C*, 1.0; *D*, 1.1; *E*, 1.9; *F*, 3.1.

In order to obtain more detailed information on this degradation process, brain cells were labeled with arginine-^{14}C for a constant time and the radioactive protein remaining was periodically determined. A more accurate measurement of the dynamics of protein turnover was obtained when sampling times were shortened to 10 min for a total of 50–70 min. Fig. 3A shows a more accurate representation of the dynamic state of protein synthesis and degradation. Incorporation of arginine-^{14}C into acid-insoluble protein continued for 10 min, which was apparently due to the lag between the addition of the excess, unlabeled arginine and the achieving of an effective intracellular concentration. After this period, there was a rapid decrease in labeled cellular protein, followed by another increase in labeled arginine incorporation. This second cycle of amino acid incorporation could have been due to the reutilization of labeled arginine from degraded protein. It can be seen, in Fig. 3A, how measurements at different time intervals would give variable results and conflicting data on absolute rates of protein turnover. Fig. 3A also emphasizes the complexity involved in measuring the actual rates of degradation in a system where reutilization can not be elminated.

Although similar studies were made on cell suspensions from 15-day brain preparations, no detectable degradation was observed. This suggested that the rate of protein turnover was less than that measured with newborn mouse brain cells. However, it was possible that the rate of degradation was similar to that of the 1-day cell suspension, since the rate of protein synthesis was considerably lower and a loss in radioactivity could have been within experimental error.

In order to eliminate the reincorporation of labeled arginine during protein degradation, translational events were inhibited by the addition of puromycin. Cellular protein was labeled with arginine-^{14}C, a concentration of puromycin sufficient to inhibit over 97% of protein synthesis (1×10^{-4} M) was added, and the loss of acid-insoluble radioactivity was measured during the reincubation period (Fig. 3B). Instead of amplifying the measured degradation of cellular protein, puromycin caused a marked stabilization of the macromolecules. This suggested that the degradation of cellular protein was, at least indirectly, coupled to translational events.

DISCUSSION

An understanding of the dynamic aspects of protein turnover is important to our understanding of neural development and differentiation. It is the balance between synthesis and degradation that determines the intracellular concentration and the persistence of proteins during developmental stages. The regulation of protein synthesis and its turnover can be important to the study of enzyme induction, regulation of protein metabolism during differentiation, as well as biological memory (2, 4, 7, 10).

Even though our initial experiments with brain cells from 1-day preparations indicated that the loss of ^{14}C from hot TCA-insoluble material was related to the amount of arginine incorporated, it soon became evident that the degradation process was complex. When time was allowed for a significant amount of incorporation and a short assay interval was used, we found that the rate of degradation fluctuated and that this fluctuation appeared to diminish with time. A similar phenomenon in Chinese hamster cells has been reported

TABLE I

The Loss of Radioactive Label from TCA-Precipitable Protein in 1-Day Old Brain Cell Suspensions Preincubated with Arginine-^{14}C or Valine-^{14}C

Time when excess amino acid added*	Specific activity at time of addition	Chase interval	Specific activity at end of chase	Loss in radioactive protein	
(min)	*(10^{-3} cpm /mg)*	*(min)*	*(10^{-3} cpm /mg)*	*Total %*	*% per min*
Valine					
		Experiment 1			
15	5.6	45	4.3	23	0.5
30	11.5	30	6.8	41	1.4
45	17.4	15	13.0	25	1.7
		Experiment 2			
15	7.8	45	6.0	23	0.5
30	16.7	30	12.0	28	0.9
45	24.2	15	23.0	5	0.3
Arginine					
15	17.3	45	15.4	11	0.4
30	37.5	30	33.8	10	0.3
45	47.3	15	52.1	−10	−0.7

* 1-day cell suspensions were incubated in the presence of arginine-^{14}C or valine-^{14}C and, at different intervals during incubation (column 1), an excess of L-arginine or L-valine was added. The suspensions were allowed to reincubate for a total time of 60 min (column 1 and column 3).

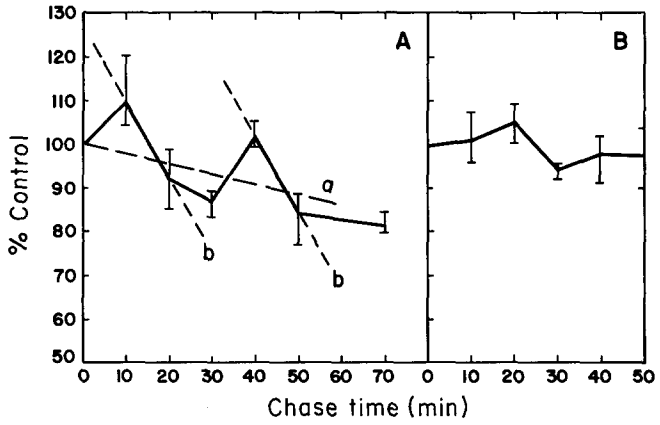

FIGURE 3 The rate of protein turnover and the influence of puromycin on protein breakdown. 1-day mouse brain cell suspensions labeled for 45 min and then reincubated in the presence of excess, unlabeled arginine. Every 10 min, duplicate reactions mixtures were assayed. Each point is the mean of six individual experiments. The bars at each point represent the range of values. At the time of reincubation, the specific activity was 4.5×10^4 cpm/mg protein. Frame A, a 1000-fold excess of unlabeled arginine was added. The slopes of lines *a* and *b* represent, respectively, a minimal and maximal measured rate of protein degradation. Frame B, with the addition of puromycin (1×10^{-4} M).

by Klevecz (8). As in Klevecz's work, the cyclic nature of specific activity of protein may be attributable to an oscillation in the specific activity of the arginine pools as a result of the release of arginine-^{14}C from highly labeled proteins.

A half-life of 3.5 hr was obtained when calculations were based on data with long sampling intervals (Fig. 3A, line a). On the other hand, only when short periods were used could the cyclic nature of protein turnover be measured. This

illustrated that the average rate of protein degradation was much more rapid than might be observed. From the latter experiments, a half-life of proteins labeled in vitro was calculated to be approximately 0.5 hr (Fig. 3A, line b). Many proteins have longer half-lives than those reported here, especially the structural proteins which have been studied in greatest detail. It is difficult, at the present time, to correlate the rates of protein turnover in brain cell suspensions with those reported with in vivo studies. The relatively short labeling periods employed in this study of protein metabolism may selectively label a specific population of proteins characterized by a rapid turnover.

The coupling of protein synthesis and degradation in several biological systems has recently been studied. The inhibition of protein synthesis by cycloheximide has been shown to stabilize the intracellular tyrosine transaminase in liver cells (1). It has also been reported that aminoacyl-tRNA influences the rate of protein breakdown in *Escherichia coli* (5). One possible mechanism for the coupling of synthesis and degradation, either directly or indirectly, may be the ratio of aminoacylated to deacylated tRNA in the intracellular pools. The inhibition of tryptophan pyrrolase activity by a deacylated species of tyrosyl-tRNA has recently been reported (6). It has been suggested that this inhibition, through feedback systems, would lead to an increase in available, free tyrosine which in turn would allow the aminoacylation of the tyrosyl-tRNA. It is possible that the rate of protein turnover in brain cells is influenced by aminoacylated and deacylated tRNA. However, a considerable amount of research will be required to substantiate the possibility.

Although it was not possible to determine specific rates of degradation for 15-day cell suspensions, it would have been possible to detect rates sufficiently high enough to account for the 10-fold net difference in protein synthesis observed. Thus, it appears that the greater rate of protein accumulation in younger brain tissue is actually a reflection of a higher rate of synthesis and not the result of a more rapid rate of degradation in the more mature preparations.

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