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Lower Rates of Protein Degradation in Developing Rat Brain

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Protein-degradation rates in developing rat brain were estimated from the decay in total radioactivity in proteins labelled by a single intraperitoneal injection of $\text{NaH}^{14}\text{CO}_3$ to 5-day-old animals. In contrast with previous reports, our results indicate that degradation rates are lower in developing than in adult brain and suggest that in brain, as has been observed in liver, adrenal gland, muscle, cultured mammalian cells and bacteria, reduced rates of protein degradation contribute to the increase in protein content under conditions of rapid growth.

The protein content of mammalian tissues is generally determined by the balance between rates of protein synthesis and degradation (Goldberg & Dice, 1974; Schimke, 1974). A variety of studies has shown reduced rates of protein degradation during periods of rapid growth. Such observations have been made in skeletal muscle (Goldberg, 1969), regenerating liver (Swick & Ip, 1974; Scornik & Botbol, 1976), developing liver (Conde & Scornik, 1977) and adrenal gland (Canick & Vilee, 1974), and also in cultured mammalian cells (Roberts & Yuan, 1974; Tauber & Reutter, 1978) and bacteria (Koch & Levy, 1955; Mandelstam, 1960). In the developing brain, however, degradation rates have been reported to be more rapid than they are in the adult animal (Stewart & Urban, 1972; Volpe *et al.*, 1973; Dunlop *et al.*, 1978). In the experiments described here, protein-degradation rates in developing brain were estimated from the decay in total radioactivity after labelling with $\text{NaH}^{14}\text{CO}_3$. Because amino acids labelled by CO_2 fixation appear to be minimally reutilized (Chee & Dahl, 1977, 1978), we believe $\text{NaH}^{14}\text{CO}_3$ to be an appropriate tracer. In contrast with previous reports, our data show that degradation rates are lower in the developing brain than they are in the brains of adult animals.

Materials and Methods

Animals employed were rats of the Sprague-Dawley strain obtained from King Animal Laboratories (Oregon, WI, U.S.A.). Mothers of suckling animals were given Lab-bloc chow (Allied Mills, Chicago, IL, U.S.A.) and water *ad lib*. $\text{NaH}^{14}\text{CO}_3$ (58.5 Ci/mol) was obtained from Amersham/Searle (Arlington Heights, IL, U.S.A.). All other reagents were of the highest purity available.

For measurement of protein-degradation rates, 5-day-old rats were injected intraperitoneally with

1 mCi of $\text{NaH}^{14}\text{CO}_3$ /100 g body wt. Groups of five to seven animals were killed at intervals from 4 h to 12 days later. Brains were rapidly removed and individually homogenized directly in 10% (w/v) trichloroacetic acid or fractionated as described previously (Chee & Dahl, 1977, 1978) and the fractions treated with an equal volume of 10% trichloroacetic acid. In certain experiments, livers were also removed and homogenized in 10% trichloroacetic acid. Precipitated proteins were collected by centrifugation, extracted with hot 5% trichloroacetic acid followed by two extractions with ethanol/diethyl ether (3:1, v/v) and a final extraction with diethyl ether. After drying overnight, the proteins were dissolved in a known volume of 88% formic acid and a portion was removed for measurement of radioactivity. Protein in another portion was measured by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. Radioactivity was measured in a Packard Tri-Carb liquid-scintillation spectrometer. The scintillation fluid consisted of a mixture of Triton X-100 and toluene (5.0 g of PPO (2,5-diphenyloxazole) and 1.2 g of dimethyl-POP [1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene] dissolved in 1.5 litre of toluene and Triton X-100 (2:1, v/v)). Counting efficiencies were determined by an automatic external standard. A minimum of 10000 c.p.m. was accumulated. Since allowance must be made for the dilution of radioactivity by the growth of the developing organs, the data are expressed in terms of total protein radioactivity.

Results and Discussion

The decay in total radioactivity in mixed brain proteins, labelled by a single intraperitoneal injection of $\text{NaH}^{14}\text{CO}_3$ to 5-day-old rats, is shown in Fig. 1(a). Similar data reported previously by this laboratory for young adult brain (Chee & Dahl, 1978) are presen-

Fig. 1. Decay in total radioactivity in proteins in developing rat brain labelled by a single intraperitoneal injection of $\text{NaH}^{14}\text{CO}_3$. The data were obtained as described in the Materials and Methods section. (a) Total mixed brain proteins; (b) soluble proteins; (c) cell-body mitochondrial proteins; (d) microsomal proteins. Each point represents the mean $\pm$ S.E.M. of the observed values obtained from five to seven rat brains. O, Decay in brain protein radioactivity reported previously by this laboratory for rats weighing 95–100 g at the time of injection (Chee & Dahl, 1978).

ted for comparison. Since the decay is not linear, the data cannot be described by a single degradation rate constant, but it is clear that the apparent rate of brain protein turnover is lower in the developing than in the adult animal. The rates of decay of total radioactivity in soluble (Fig. 1b), cell-body mitochondrial (Fig. 1c) and microsomal (Fig. 1d) proteins

were also determined. These are compared with previously reported decay rates in subcellular fractions of young adult rat brain expressed in terms of specific radioactivity of acidic amino acids (Chee & Dahl, 1978). There is no statistically significant difference between decay in specific radioactivity of intact protein and decay in specific radioactivity of

acidic amino acids isolated from protein hydrolysates in either young adults (Chee & Dahl, 1978) or developing rats (J. L. Dahl & V. J. Cummings, unpublished observations). The differences between the degradation rates in developing and young adult animals are particularly striking in the soluble and microsomal fractions. Proteins in the soluble fraction of developing rat brain appear to turn over at half the rate ($t_{1/2} \approx 7$ days) observed in the adult animal ($t_{1/2} \approx 3$ days). There is almost a 4-fold difference between the short-term decay rates in microsomal proteins in the adult and developing brain. Although the results are not shown, decay in total radioactivity in the crude mitochondrial (P_2) fraction from developing brain was also measured; there was a minimal loss in total radioactivity over the 12-day time course of measurement. This crude mitochondrial fraction presents a heterogeneous population, including mitochondria, lysosomes, synaptic endings, plasma membranes, myelin and axonal fragments. The protein content of this fraction increased at a greater rate during postnatal development than did that of the other fractions studied. Although decay rates for the P_2 fraction in adult brain are not available for comparison, half-lives of synaptic proteins range from 6 to 9 days (Chee & Dahl, 1978).

The decay in total radioactivity from liver proteins of developing rats labelled with $\text{NaH}^{14}\text{CO}_3$ was also measured. The results were very similar to those obtained by Conde & Scornik (1977) in their study of degradation rates in neonatal mouse liver. A half-life of 5 days was estimated from the apparent linear decay between days 2 and 12. These results confirm their observation that the growing livers of newborn animals degrade protein more slowly than do adult livers.

Our finding of an apparent lower rate of degradation in developing rat brain is in contrast with previous reports. Using $[^{14}\text{C}]$ leucine as precursor, Volpe *et al.* (1973) had observed that the rate of degradation of soluble brain proteins was accelerated in suckling animals as compared with adults, i.e. that the average half-life in the adult rat was 8.7 days and that in the neonatal animal 4.5 days. They also found that the degradation rate of fatty acid synthetase was more rapid in the developing brain. It may be important that they followed decay after labelling brain proteins with radioactive leucine. Although we have observed that leucine is subject to extensive reutilization in the young adult (Chee & Dahl, 1978), it is possible that reutilization of this label is less in developing brain. With leucine as precursor, Volpe *et al.* (1973) also observed little difference between protein-degradation rates in the developing and adult rat liver, whereas Conde & Scornik (1977), who used $\text{NaH}^{14}\text{CO}_3$ to label protein, found that neonatal mouse liver degrades protein more slowly than does adult liver. The advantages

of $\text{NaH}^{14}\text{CO}_3$ as precursor for the measurement of degradation rates in liver have been detailed by Swick & Ip (1974). Previous work in our laboratory has shown that in brain also precursor reutilization is minimal when decay in radioactivity is measured after labelling protein with $\text{NaH}^{14}\text{CO}_3$ (Chee & Dahl, 1977, 1978). Those results were obtained in experiments with young adult animals; presumably, they would hold for developing brain as well. The lower decay rates we have observed in neonatal brain could reflect greater reutilization of label by rapidly growing developing brain than by slowly growing adult tissue. However, our results are in accord with the findings of Marks *et al.* (1975), who observed lower proteinase activity in immature than in adult brain.

Dunlop *et al.* (1978) estimated rates of protein degradation in several regions of developing rat brain by subtracting the rate of accumulation of protein from the rate of protein synthesis. Their data show that the average rate of degradation is higher in the developing nervous system than in that of adult animals, and suggest that protein accumulation in developing brain results from a relatively small difference between a rapid rate of degradation and an even more rapid rate of synthesis. Stewart & Urban (1972) used a similar calculation in their report of a more rapid degradation of S-100 protein in developing than in adult brain. Dunlop *et al.* (1978) have discussed the limitations of this method of estimating protein-degradation rates. Not only is there uncertainty associated with the measurement of protein-synthesis rates *in vivo*, but it is difficult to estimate the rate of protein accumulation accurately, since daily changes in total protein content are small. Nevertheless, Conde & Scornik (1977) found that both direct (measurement of decay after pulse labelling) and indirect (calculation from synthesis rates) methods showed reduced rates of protein degradation in developing mouse liver. The reason that the two methods give contradictory estimates of protein-degradation rates in developing brain is not immediately obvious.

In summary, the results presented in this report show a reduced rate of protein degradation during the period of rapid growth in developing brain. Our results suggest that in brain, as has been observed in other organs (Goldberg, 1969; Swick & Ip, 1974; Canick & Villet, 1974; Scornik & Botbol, 1976; Conde & Scornik, 1977), in cultured mammalian cells (Roberts & Yuan, 1974; Tauber & Reutter, 1978) and in bacteria (Koch & Levy, 1955; Mandelstam, 1960), a reduced rate of protein degradation is a feature of enhanced growth.

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