Brain Research, 54 (1973) 129–142

SYNTHESIS OF SOLUBLE NEURONAL PROTEINS *IN VIVO*. AGE-DEPENDENT DIFFERENCES IN THE INCORPORATION OF LEUCINE AND PHENYLALANINE

D. E. JOHNSON* AND O. Z. SELLINGER

Laboratory of Neurochemistry, Mental Health Research Institute, University of Michigan Medical Center, Ann Arbor, Mich. 48104 (U.S.A.)

(Accepted October 25th, 1972)

SUMMARY

[^{3}H]Leucine and [^{3}H]phenylalanine were injected intracerebrally into 8- and 18-day-old rats and, in addition, [^{3}H]proline was injected into 8-day-old rats only. Neuronal perikarya were isolated from the cerebral cortex 15 min later and the rapidly labeled proteins recovered in the high-speed soluble supernatant fraction were analyzed by polyacrylamide disc gel electrophoresis.

Using sodium dodecyl sulfate (SDS)–urea gels, significant differences were noted between the [^{3}H]leucine and the [^{3}H]phenylalanine-labeled proteins of the 8-day-old neuron, the former consisting largely of slow-moving, high molecular weight species, while the latter consisted of fast-moving, small molecular weight species. The pattern of the [^{3}H]proline-labeled proteins conformed generally to that of [^{3}H]-phenylalanine.

The trend seen in the 8-day-old neurons was largely reversed in the 18-day-old neurons, demonstrating that the protein synthetic machinery of the cortical neuron undergoes qualitative, precursor-dependent adjustments during its development.

Under the conditions of our experiments, no labeling of microtubule proteins was detected. Furthermore, no radioactivity migrating as microtubule protein was found in the vinblastine precipitate of the neuronal soluble proteins.

Electrophoresis of a hypotonic extract of the neuronal soluble proteins revealed a number of additional radioactive proteins, presumed to be nascent polypeptides caught in the lumen of the cisternae of the endoplasmic reticulum, while *en route* to extraperikaryal destinations.

* Post-doctoral trainee. Present address: Division of Neuroscience, University of California at San Diego, La Jolla, Calif., U.S.A.

INTRODUCTION

Even though proteins account for about 40% of the adult brain weight[14], a proportion remaining practically unchanged in states of severe protein deficiency[12,13], little is known about changes in protein quality during the ontogenetic development of the nerve cell. Recently, Grossfeld and Shooter[6] reported that in mouse brain the most pronounced rate of protein accumulation coincides with the highest frequency of compositional alterations in their structure in the early postnatal period, while we reported significant precursor- and age-dependent differences in *in vivo* rates of protein synthesis in the cerebral cortex of the rat[20].

In the recent past our laboratory has addressed itself to the general question of the diversity of synthetic patterns among cerebral proteins during development by comparing (a) the time course of synthesis of nascent chains *in vivo* in immature nerve and glial cells[7]; (b) the ability of immature neuronal cell bodies to carry out protein synthesis *in vitro* under a number of different experimental conditions[9]; (c) the incorporation of [^{14}C]phenylalanine into neuronal and glial proteins *in vivo*[8,18] as a function of early postnatal age; and (d) the selective inhibitory effects on cerebral protein synthesis of the convulsant agent methionine sulfoximine[17].

In the present study we compare the incorporation *in vivo* of different amino acids into a selected family of neuronal proteins, namely those which become labeled in 15 min following an intracerebral pulse of radioactive amino acid and which, in addition, are recovered in the high-speed soluble fraction of a differentially fractionated neuronal homogenate.

METHODS AND MATERIALS

Sprague–Dawley 8- and 18-day-old male rats were used. L-[U-^{14}C]Phenylalanine with a specific activity of $>$ 350 mCi/mmole, L-[2,3-^{3}H]proline with a specific activity of $>$ 5 Ci/mmole, L-[^{3}H]phenylalanine with a specific activity of 2–5 Ci/mmole and L-[4,5-^{3}H]leucine with a specific activity of 5 Ci/mmole were obtained from New England Nuclear Corporation (Boston, Mass.). Bovine serum albumin (BSA, fraction V) was obtained from Pentex Biochemicals (Kankakee, Ill.), polyvinylpyrrolidone (PVP; Plasdone C, average mol. wt.: 40,000) was purchased from GAF Corporation (Calvert City, Ky.), Ficoll was obtained from Pharmacia (Uppsala, Sweden), sucrose (special enzyme grade) was purchased from Mann Research Laboratories (New York, N.Y.), 2,5-diphenyloxazole (PPO) and 1,4-bis-(5-phenyloxazolyl-2)-benzene (dimethyl POPOP) were obtained from Packard Instruments (Downers Grove, Ill.), Biosolv-3 was purchased from Beckman Company (Fullerton, Calif.), and vinblastine sulfate was obtained from Eli Lilly and Company (Indianapolis, Ind.). All other reagents were of the purest grade available. Millipore filters (GA-6, 0.45 μm pore size) were obtained from Gelman Instrument Company (Ann Arbor, Mich.). Nylon bolting cloth was purchased from Tobler, Ernst and Traber (Elmsford, N.Y.).

Injecting procedure and isolation of neuronal perikarya

Labeling of the neuronal soluble proteins *in vivo* was accomplished by a single

intracerebral injection to lightly anesthetized 8- and 18-day-old rats of 2.5 or 6.25 μCi, respectively, of L-[U-^{14}C]phenylalanine, L-[4,5-^{3}H]leucine, L-[2,3-^{3}H]proline or L-[^{3}H]phenylalanine. After 15 min the animals were killed by decapitation. The details of the procedures of isolation of neuronal perikarya[16] and of their subcellular fractionation in 0.25 *M* sucrose to obtain the fraction containing the soluble (non-sedimentable) proteins, of the measurement of radioactivity and of the determination of protein and RNA were reported previously[7,8,19]. When required, a homogenate in 0.25 *M* sucrose of whole brain cortex was fractionated alongside the neuronal perikarya and the high-speed soluble fraction obtained by subjecting the post-mitochondrial supernatant of both preparations to 104,000 × *g* for 60 min was used for electrophoresis (see below).

Polysomal profiles

Neuronal perikarya from ten 8-day-old male rats[7,9] were isolated as two separate pellets, one of which was homogenized in 0.25 *M* sucrose and the other in 5 m*M* Mg acetate, 20 m*M* Tris (pH 7.2) and 25 m*M* KCl (medium TKM). The subcellular fractionation of the neuronal perikarya was carried out as previously reported [7,8]. Equal volumes of the nuclear and the microsomal fractions were layered on a 35–60% (w/v) linear sucrose gradient and were centrifuged for 14.5 h at 49,000 × *g* in the SW-41 rotor of the L2-50 Spinco ultracentrifuge. For collection of the gradients, the bottom of the centrifuge tube was pierced and the gradients pumped at a constant rate through a rectangular flow-cell (0.4 cm path length, Uvicord LKB Instrument) while being continuously monitored at 254 nm[19]. Crystalline bovine serum albumin (50 μg) was added to each tube and the radioactivity in the effluent determined as previously described[7,8].

Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis of the neuronal and cortical soluble proteins was carried out at room temperature. Stock and working solutions were those recommended by Davis[1]. Three types of gel system were used: (a) the original gel system of Davis[1], (b) a modified system containing 8 *M* urea and 0.1% sodium dodecyl sulfate (SDS) in the gel and 0.1% SDS in the buffer[21], and (c) as (b), but without SDS. Electrophoresis was conducted at a current varying from 2 mA/tube at the beginning to a maximum of 5 mA/tube during the course of the run. To allow for direct comparison between gels, closely similar amounts of radioactivity were placed on the gels in all cases*. When resorted to, staining was at least for 1 h in a 0.2% (w/v) solution of Coomassie brilliant blue in methanol–glacial acetic acid–water (5:1:5, v/v) and destaining was accomplished by several changes of 7.5% (v/v) acetic acid. The radioactive gels were sectioned after freezing them in a slurry of acetone–dry ice, with the aid of a commercial gel slicer and the approximately 35 slices were placed in scintilla-

* Since preliminary experiments indicated no detectable difference in the staining pattern of the neuronal soluble protein fraction at 8 and 18 days, our results are presented mainly in terms of differences in the radioactive patterns obtained under the different experimental conditions. Also, as shown in Fig. 6, the presence of bovine serum albumin[9,16] components interfered with the optimal interpretation of the stained gels.

tion vials containing 1 ml of Biosolv-3. The capped vials were incubated at 55 °C for 16–18 h whereupon 10 ml of scintillation solvent[7,9] was added and the radioactivity determined as previously described[7,9].

Vinblastine precipitation

Vinblastine sulfate was added to the 104,000 × *g* supernatant to a final concentration of 2×10^{-3} *M* and, in most experiments, $MgCl_2$ was then added to a final concentration of 2.5 m*M*. The solution was allowed to incubate at 2–4 °C for 30 min. After centrifuging at 27,000 × *g* for 15 min the supernatant was removed and the pellet resuspended and recentrifuged as above twice more. The final pellet was used for electrophoresis in gel system (b)[21].

Preparation of microtubule protein

Microtubule protein was prepared from rat brain cortex by the DEAE Sephadex batch-precipitation method of Weisenberg *et al.*[25].

RESULTS

General remarks about the electrophoretic analysis of the radioactive proteins

The radioactive polyacrylamide gel band patterns shown in Figs. 1–5 below are representative of the sum total of the results obtained in our study. We carried out 3 separate experiments at each of the two ages using [^{3}H]leucine and [^{3}H]phenylalanine and a single experiment using [^{3}H]proline. In Figs. 1 and 3 the neuronal profiles (panels A, C, and E) and the whole cortex profiles (panels B, D and F) were obtained from high-speed soluble supernatants prepared in parallel from different groups of rats. In each experiment we ran a sample each of the neuronal and of the whole cortex supernatants in the 3 different electrophoretic systems described in Methods. In Figs. 2 and 4 the same design was followed except that only the results of the runs using electrophoretic systems (a) and (b) (see Methods) are shown. In our experience there was very little run-to-run variation in the labeling profiles, since our conditions of injecting the animals, of cell and fraction isolation and of sample preparation were rigidly controlled from experiment to experiment. It should also be noted that, for simplicity, the upper left panel of Fig. 7 and panel C of Fig. 1 depict one and the same electrophoretic profile.

Polyacrylamide gel electrophoresis of labeled neuronal and cortical soluble proteins

The radioactive profiles of the [^{3}H]leucine-labeled soluble proteins isolated from the neurons and the whole cortex of 8-day-old rats are shown in Fig. 1. Gel system (a) gave one large radioactive area (panels A and B) whereas gel system (b) revealed 7 radioactive peaks in the neurons (panel C) and 3 radioactive peaks in the whole cortex (panel D) one of which was particularly highly labeled. The labeled neuronal proteins in system (b) were mostly high to medium molecular weight molecules whereas the labeled cortical materials were predominantly medium to low molecular weight molecules.

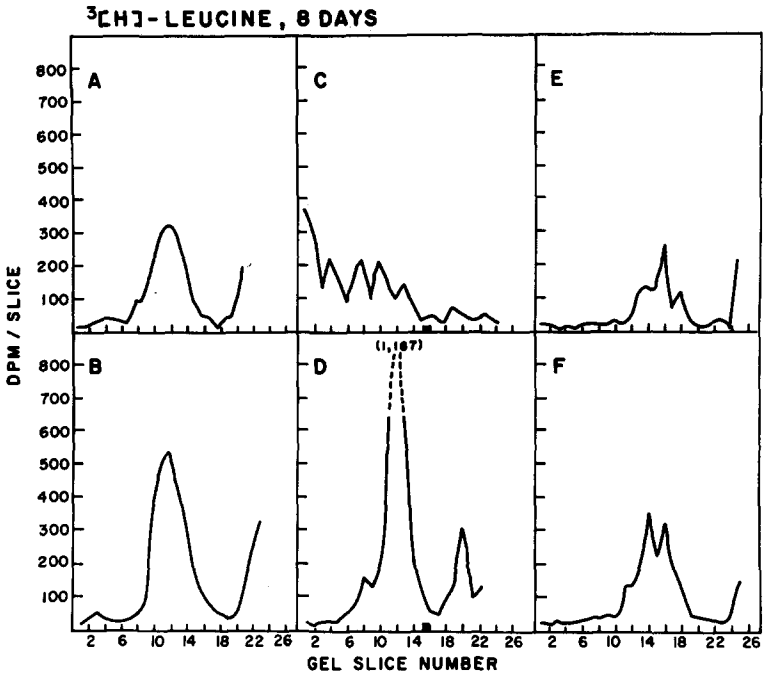

Fig. 1. Electrophoresis of neuronal and cortical soluble proteins. [^{3}H]Leucine (2.5 μCi/rat) was injected intracerebrally into 8-day-old rats which were killed 15 min later. Neuronal cell bodies were isolated from the cerebral cortex[7,16] and the soluble fraction obtained by high-speed centrifugation[7] was used for electrophoresis (see Methods). The neuronal profiles are shown in panels A, C and E. In A, the system of Davis[1] was used, in C the gel contained 0.1 % SDS and 8 *M* urea[21] and the buffer 0.1 % SDS, and in E the gel contained 8 *M* urea only. Panels B, D and F show the radioactivity profiles of the soluble cortical proteins run in the same gel systems. See text for operational details. The direction of electrophoresis was from left to right. ■, Microtubule protein marker[25].

The radioactive profiles of the [^{3}H]leucine-labeled neuronal and cortical soluble proteins of 18-day-old rats are shown in Fig. 2. Gel systems (a) and (b) gave about the same number of radioactive peaks. The neuronal proteins were of intermediate molecular weight (panel C) while the spectrum of the cortical proteins (panel D) consisted of low, medium and high molecular weight species, the former forming a highly labeled migration front.

Fig. 3 shows the radioactive profiles of the [^{3}H]phenylalanine-labeled neuronal and cortical soluble proteins isolated from 8-day-old rats. Five radioactive peaks were noted in gel system (b) (panels C and D). The high and medium molecular weight molecules were only moderately labeled in this system; conversely, the low molecular weight species were appreciably labeled, irrespective of the gel system used.

The radioactive profiles of the [^{3}H]phenylalanine-labeled soluble proteins of the 18-day-old rats (Fig. 4) show 4 radioactive neuronal peaks (panel C) and about 6 cortical peaks. Both profiles consisted of a mixture of molecular weight species.

The radioactive profiles of the [^{3}H]proline-labeled soluble proteins in 8-day-old

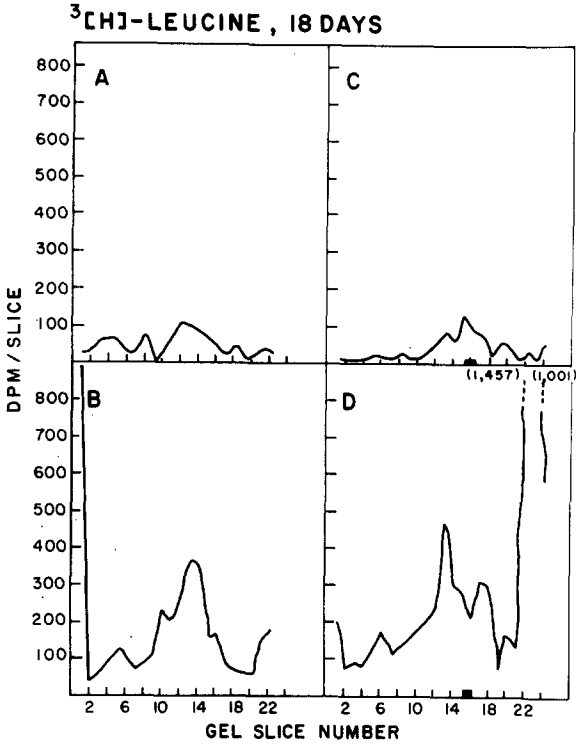

Fig. 2. Electrophoresis of neuronal and cortical soluble proteins. [^{3}H]Leucine (6.25 μCi/rat) was injected intracerebrally into 18-day-old animals which were killed 15 min later. The remainder of the procedure was as described in the legend to Fig. 1. Panels A and C refer to the radioactivity profiles of the neuronal proteins while panels B and D refer to the profiles of the cortical proteins. In panels A and B the gel system of Davis[1] was used and in panels C and D, gels containing 0.1% SDS and 8 *M* urea were used. ■, Microtubule protein marker[25].

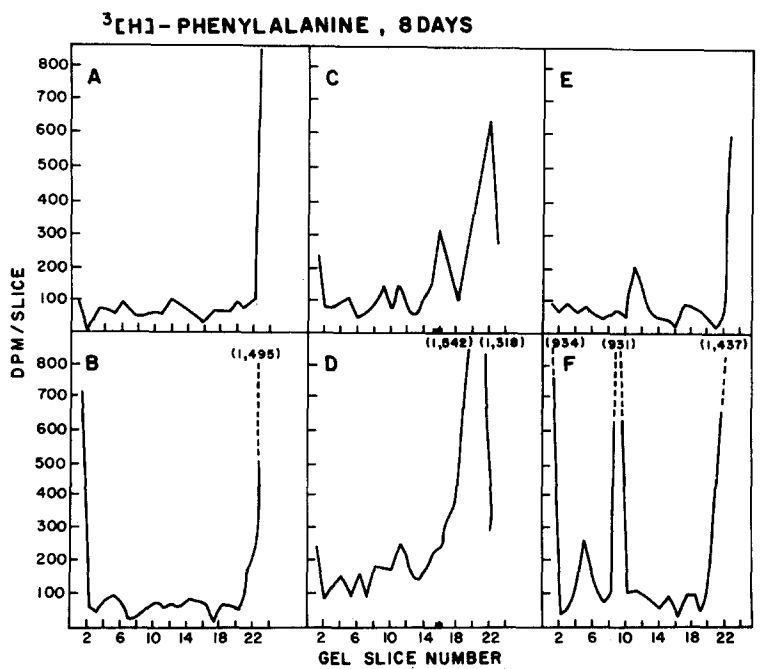

Fig. 3. Electrophoresis of neuronal and cortical soluble proteins. [^{3}H]Phenylalanine was injected into 8-day-old rats. All other conditions were as described in the legend to Fig. 1. ■, Microtubule protein marker[25].

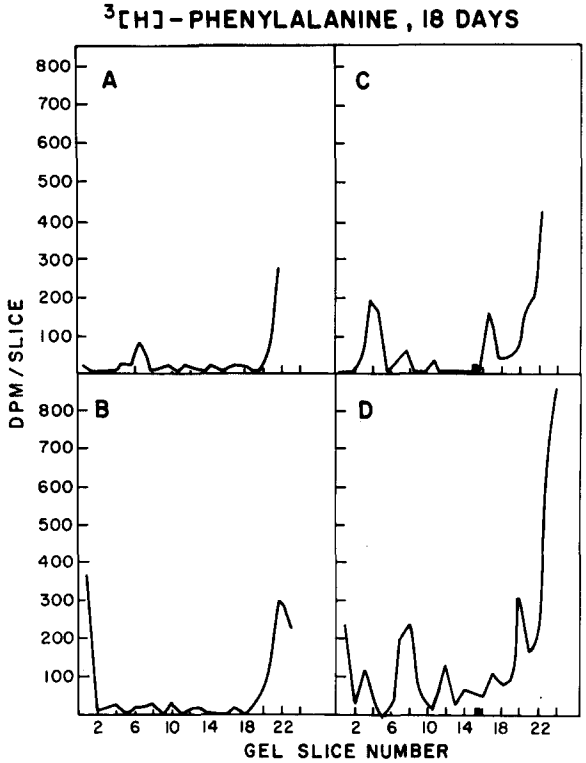

Fig. 4. Electrophoresis of neuronal and cortical soluble proteins. [^{3}H]Phenylalanine was injected into 18-day-old rats. All other conditions were as described in the legend to Fig. 2. ■, Microtubule protein marker[25].

rats (Fig. 5) revealed a heterogeneous pattern in the neurons and in the whole cortex, the low molecular weight species being labeled preferentially.

When gels containing 8 *M* urea and no SDS were used (system (c) see Methods), the electrophoretic patterns showed a larger number of radioactive peaks and a better resolution of radioactivity than in system (a) (Figs. 1 and 3, panels E and F), but fewer peaks and a generally poorer resolution than when system (b) was used. Of particular significance was the sharp peak in gel slice number 9 when [^{3}H]phenylalanine and 8-day whole cortex supernatant were used (Fig. 3, panel F).

Precipitation of the rapidly labeled soluble proteins with vinblastine

Vinblastine sulfate was added to the neuronal high-speed soluble supernatant and the radioactivity recovered in the precipitate and in the supernatant obtained by centrifugation of the resulting suspension (see Methods) was determined. Protein assay revealed that only about 10–15% of the proteins of the parent fraction were recovered in the vinblastine precipitate. Electrophoresis of the vinblastine precipitate in 8 *M* urea–SDS gels (system (b), see Methods) resulted in several protein bands,

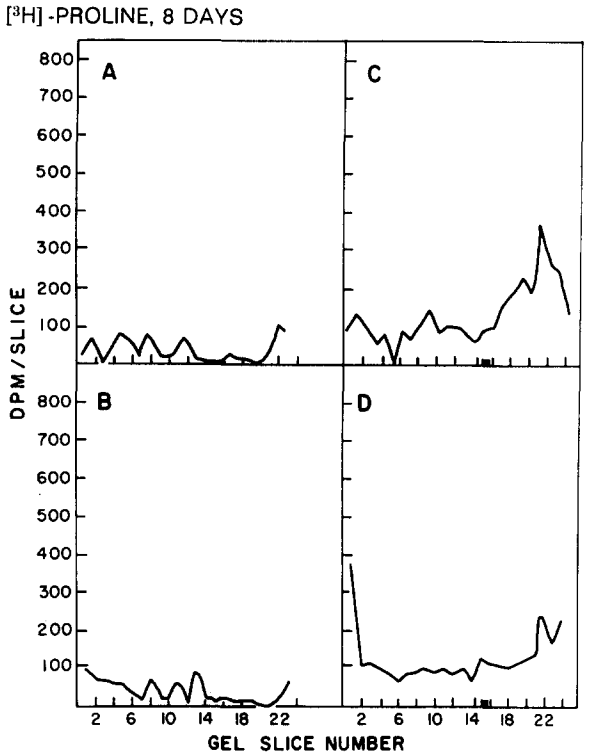

Fig. 5. Electrophoresis of neuronal and cortical soluble proteins. [^{3}H]Proline was injected into 8-day-old rats. All other conditions were as described in the legend referring to panels A–D of Fig. 1. ■, Microtubule protein marker[25].

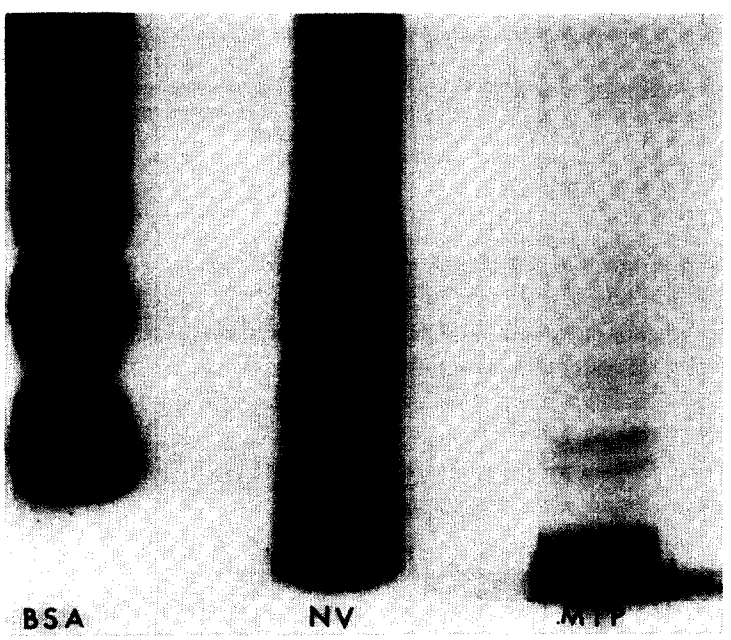

Fig. 6. Electrophoresis of vinblastine-precipitated neuronal proteins: stained gels. The gel in the middle (NV) is of the vinblastine precipitate of the neuronal high-speed soluble supernatant (see Methods); the gel to the right (MTP) is of authentic microtubule protein isolated from the cerebral cortex of 21-day-old rats[25] showing the characteristic double banding; and the gel to the left (BSA) is of fraction V bovine serum albumin. Note the absence of microtubule protein bands in gel NV. The direction of migration is from top to bottom.

TABLE I

THE PRECIPITATION OF RADIOACTIVE NEURONAL SOLUBLE PROTEINS BY VINBLASTINE

[^{14}C]Phenylalanine was injected in experiment No. 1 and [^{3}H]leucine in experiment No. 2. 8-day-old animals were used. The duration of the radioactive pulse was 15 min. In both experiments the precipitation by vinblastine was carried out in the presence of 2×10^{-3} *M* $MgCl_2$. The use of different amino acids in the two experiments was purely for illustrative purposes and no conclusions as to their relative efficiencies in synthesis should be drawn from the different enrichment factor values.

	Specific radioactivity (disint./min/mg of protein)	*Enrichment factor**
Experiment No. 1		
104,000 × *g* supernatant	2,800	
Vinblastine precipitate	16,370	5.85
Experiment No. 2		
104,000 × *g* supernatant	3,200	
Vinblastine precipitate	40,000	12.5

* The enrichment factor refers to the ratio of the specific radioactivity of the vinblastine precipitate to that of the parent supernatant.

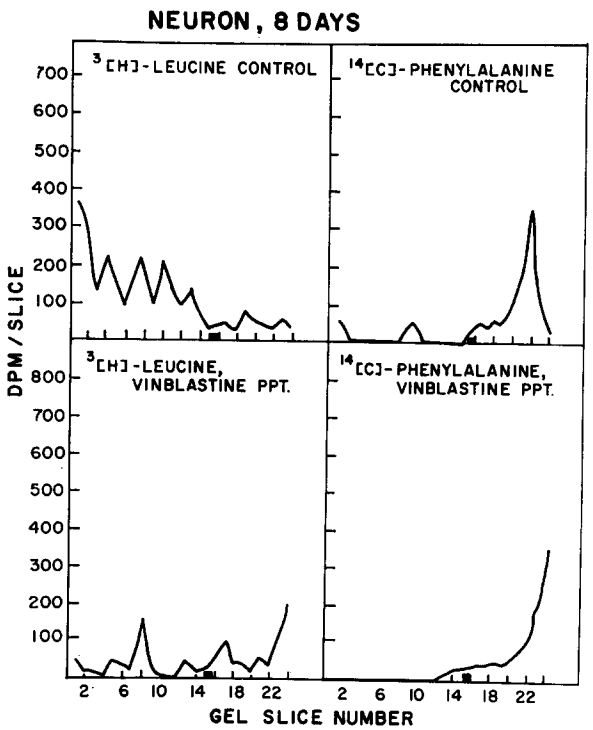

Fig. 7. Electrophoresis of vinblastine-precipitated neuronal proteins: radioactive gels. [^{3}H]Leucine or [^{14}C]phenylalanine were injected intracerebrally into 8-day-old rats, as indicated, and the neuronal cell bodies were isolated from the cerebral cortex[16] 15 min later. The upper two panels show the radioactivity profiles of the high-speed soluble supernatant fraction in system (b) (see Methods) before vinblastine, while the lower two panels depict the radioactivity profiles of the vinblastine precipitate. Note the virtual absence of radioactivity co-migrating with authentic microtubule protein (■).

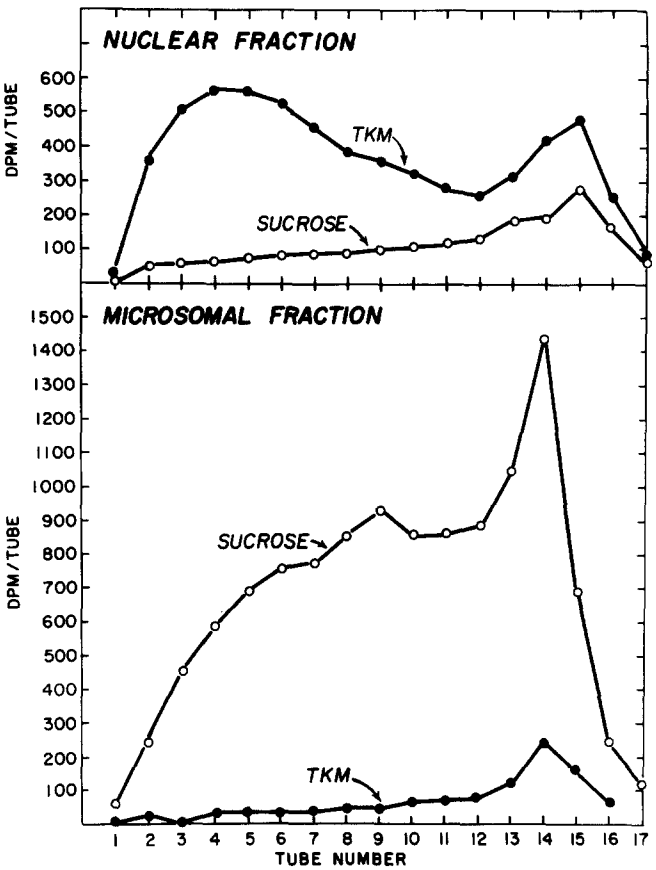

Fig. 8. The effect of a hypotonic homogenization medium on the intracellular localization of the nascent radioactivity. 8-day-old rats were injected with [^{14}C]phenylalanine (see Methods) and the neuronal cell bodies were isolated from the cerebral cortex[16] 15 min later. Homogenates of the cell bodies in 0.25 *M* sucrose and in medium TKM (see Methods) were fractionated according to the centrifugation schedule described by Johnson and Sellinger[7] and the nuclear and microsomal fractions were isolated. Suspensions of the two fractions were subjected to centrifugation on a linear gradient (35–60%) of sucrose as described in Methods and the radioactivity in each effluent tube was determined[7,19]. The bottom of the gradient is in tube 1.

none of which appeared to co-migrate with microtubule protein standards, as indicated by the stained gels shown in Fig. 6, although some appeared to co-migrate with some of the protein components of bovine serum albumin fraction V (see Materials) which is used, in conjunction with 10 m*M* Ca^{2+}, in the preparation of the neuronal perikarya[16]. The middle gel of Fig. 6 thus shows that some of the bovine serum albumin components contained in the neuronal high-speed soluble supernatant became entrained by the vinblastine-precipitated materials or were themselves precipitated by vinblastine, thus confirming the findings of Wilson *et al.*[27]. A semi-quantitative evaluation of the extent of vinblastine action is provided by the results shown in Table I in which the specific radioactivity of the neuronal high-speed soluble super-

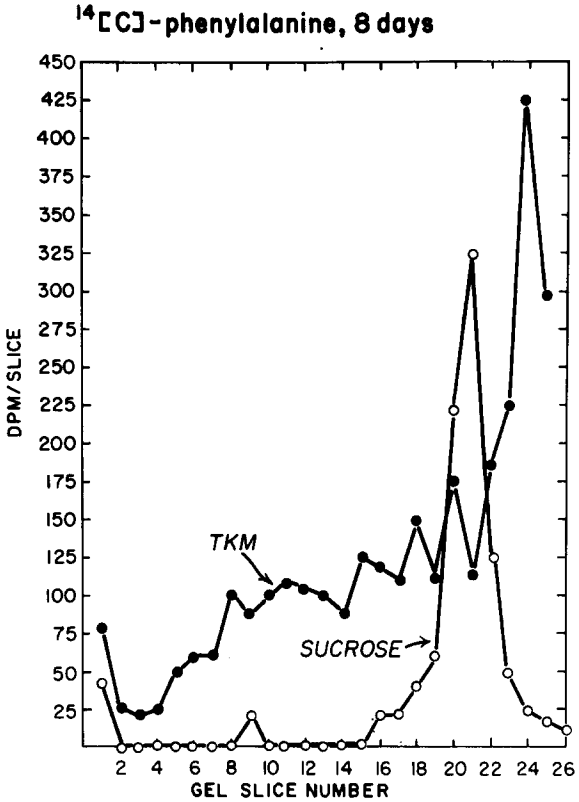

Fig. 9. Electrophoresis of the neuronal soluble proteins extracted by 0.25 *M* sucrose and medium TKM. [^{14}C]Phenylalanine was injected intracerebrally into 8-day-old rats (see Methods) and the neuronal cell bodies isolated from the cerebral cortex[16] 15 min later. The high-speed soluble supernatant fraction obtained from homogenates prepared in 0.25 *M* sucrose and medium TKM (see Methods) was subjected to electrophoresis in SDS–urea gels (system b, Methods) to give the radioactivity profiles indicated. The direction of electrophoresis was from left to right.

natant labeled, in two separate experiments, with [^{14}C]phenylalanine and [^{3}H]leucine, are compared to the specific radioactivities of the respective vinblastine precipitates. In both experiments, the vinblastine precipitate concentrated the radioactivity. Yet, as indicated by the electrophoretic profiles shown in the lower panels of Fig. 7, we were unable to identify microtubule protein among the radioactive materials precipitated by vinblastine.

Electrophoresis of the neuronal proteins extracted by a hypotonic medium

The rapidly labeled neuronal soluble proteins isolated as a high-speed supernatant in 0.25 *M* sucrose were compared to an operationally different class of proteins obtained by extraction of the neuronal cell bodies in medium TKM (see Methods). Fig. 8 shows that the extraction in this medium did not cause the release of any of the polysome-bound radioactivity into the high-speed soluble supernatant (Fig. 8, lower panel, sucrose) but that, instead, it caused the shift of the polysomes into the nuclear

fraction (Fig. 8, upper panel TKM). This finding was confirmed by parallel determinations of the amounts of RNA among the subcellular fractions isolated in the TKM and the sucrose media. Electrophoresis of the proteins extracted in the TKM medium in gel system (b) (see Methods) revealed higher levels of radioactivity and a more qualitative diversity of labeled species than was found in the sucrose extracts (Fig. 9), suggesting that the TKM medium extracted labeled materials from within the cisternal lumen of the endoplasmic reticulum membranes[15].

DISCUSSION

The present results document the age- and precursor-dependent synthesis of different rapidly labeled, soluble proteins by the neuronal cell bodies of the cerebral cortex of 8- and 18-day-old rats. The proteins labeled by [^{3}H]phenylalanine, [^{3}H]-leucine and [^{3}H]proline disclosed significant age-dependent differences in their electrophoretic migration patterns. We have shown elsewhere that the intensity of the labeling of these and of particulate neuronal proteins varies both as a function of age and precursor, both *in vivo*[8] and *in vitro* (Sellinger and Ohlsson, unpublished observation). The present findings extend the observations of Grossfeld and Shooter[6] who reported significant qualitative differences between the proteins extracted from the brain of the mouse, pre- and early postnatally. Inspection of the electrophoretic profile obtained from the neurons of the 8-day-old rats (Fig. 1, panel C) reveals that while the incorporation of [^{3}H]leucine proceeded mainly into high to medium molecular weight materials that of [^{3}H]phenylalanine (Fig. 3, panel C) was preferentially directed toward the synthesis of fast-moving, low molecular weight molecules. The electrophoretic profiles of the 18-day-old rats reflected changing precursor dependencies, for the leucine pattern shifted toward the synthesis of low to medium molecular weight species (Fig. 2, panel C), while the phenylalanine pattern remained the same as that of the 8-day-old neurons (Fig. 4, panel C). The incorporation of [^{3}H]proline (Fig. 5) conformed, in general, to that of [^{3}H]phenylalanine (Fig. 3), with the important difference that there was no fast-migrating, highly radioactive, low molecular weight component. Previously, Friedman and Wenger[4] noted significant qualitative differences among the cerebral proteins of young and adult chicks and, more recently, these workers reported an accumulation of an organ-specific 'adult type' protein in the chick brain during the first 12 days of embryonic development[5]. Studies by Warecka[23, 24] and her colleagues have also shown the sequential appearance, in the fetal human brain, of proteins 'in relation to the occurrence of various histological tissue components in the brain'. Thus, a 'brain-specific glycoprotein could be identified in fetal brains of the age of approximately 24–28 weeks and in all brains above this age'. In a related study, Piatigorsky *et al.*[10] observed specific changes in the electrophoretic pattern of newly synthesized proteins in the lens epithelial cells both as a function of cultivation time *in vitro* as well as *in vivo* and these workers suggested that their finding represented evidence that the quantitative 'proportion of polypeptides synthesized' changes as a function of age.

In agreement with the findings of Grossfeld and Shooter[6] who found that hypo-

tonic media extracted more protein from mouse brain than 0.25 *M* sucrose, we also found that the sucrose-free TKM medium extracted more radioactive neuronal protein than did 0.25 *M* sucrose alone (Fig. 9). Presumably, the hypotonic neuronal extract contained newly synthesized radioactive materials caught in the lumen of the cisternae of the endoplasmic reticulum while *en route* to extraperikaryal destinations[15].

In an attempt to characterize the rapidly labeled neuronal soluble proteins, we added vinblastine sulfate to their 104,000 × *g* high-speed soluble supernatant. Since the electrophoretic analysis of the composition of the neuronal vinblastine precipitate failed to identify microtubule protein (Fig. 7), we conclude that this protein does not become labeled in the cerebral cortex of the 8-day-old rat within 15 min of the administration of an intracerebral pulse of either [^{14}C]phenylalanine or [^{3}H]leucine. In view of the recent report[11] that the amount of microtubule protein in the cerebral cortex (of the rabbit) actually decreases during the first postnatal week, it is conceivable that we might have detected radioactivity in this protein, had we used younger animals. At any rate, the present results are in support of the observation of Dutton and Barondes[2] and of Feit *et al.*[3] who noted different time-courses of microtubule synthesis in the brains of growing and adult rats and they also provide confirmatory evidence for the lack of specificity of vinblastine as an exclusive precipitant of microtubule protein. Indeed, as shown by Wilson *et al.*[27] and as illustrated graphically in Fig. 6, vinblastine effectively precipitated some components of commercial fraction V bovine serum albumin.

In summary, the present findings document the rapidly changing precursor-dependent adjustments of the protein synthetic machinery in the developing cortical neuron. We believe that a rather precise knowledge of the modalities governing these adjustments is necessary for the understanding of the mechanisms which mediate the integration of the biochemical processes of the neuron with its specific functions. In our estimation the importance of the precursor–age synchrony as a determinant of ordered neuronal development has been largely overlooked[22,26].

ACKNOWLEDGEMENTS

Supported by U.S. Public Health Service Grants NS-06294 and MH-07417.

REFERENCES

1 DAVIS, B. J., Disc electrophoresis. II. Method and application to human serum proteins, *Ann. N.Y. Acad. Sci.*, 121 (1964) 404–427.
2 DUTTON, G. R., AND BARONDES, S., Microtubular protein: synthesis and metabolism in developing brain, *Science*, 166 (1969) 1637–1639.
3 FEIT, H., DUTTON, G. R., BARONDES, S., AND SHELANSKY, M. L., Microtubule protein: identification in and transport to nerve endings, *J. Cell Biol.*, 51 (1971) 138–147.
4 FRIEDMAN, H. P., AND WENGER, B. S., Adult brain antigens demonstrated in chick embryos by fractionated antisera, *J. Embryol. exp. Morph.*, 13 (1965) 35–43.
5 FRIEDMAN, H. P., AND WENGER, B. S., Accumulation of an organ-specific protein during development of the embryonic chick brain, *J. Embryol. exp. Morph.*, 23 (1970) 289–297.

6 GROSSFELD, R. M., AND SHOOTER, E. M., A study of the changes in protein composition of mouse brain during ontogenetic development, *J. Neurochem.*, 18 (1971) 2265–2277.
7 JOHNSON, D. E., AND SELLINGER, O. Z., Protein synthesis in neurons and glial cells of the developing rat brain: an *in vivo* study, *J. Neurochem.*, 18 (1971) 1445–1460.
8 JOHNSON, D. E., AND SELLINGER, O. Z., Age-dependent utilization of phenylalanine for the synthesis of neuronal and glial proteins, *Neurobiology*, in press.
9 KOHL, H. H., AND SELLINGER, O. Z., Protein synthesis in neuronal perikarya isolated from cerebral cortex of the immature rat, *J. Neurochem.*, 19 (1972) 699–711.
10 PIATIGORSKY, J., WEBSTER, H. DE F., AND CRAIG, S. P., Protein synthesis and ultrastructure during the formation of embryonic chick lens fibers *in vivo* and *in vitro*, *Develop. Biol.*, 27 (1972) 176–189.
11 REDBURN, D. A., AND DAHL, D. R., Changes in amount of colchicine-binding protein (tubulin) in rabbit brain during development, *J. Neurochem.*, 18 (1971) 1689–1694.
12 RICHTER, D., Protein metabolism in the brain. In F. BRUCKE (Ed.), *Biochemistry of the Central Nervous System*, Pergamon Press, London, 1958, pp. 173–184.
13 RICHTER, D., Protein metabolism in pathological states. In H. PEETERS (Ed.), *Protides of the Biological Fluids, Vol. 13*, Elsevier, Amsterdam, 1965, pp. 137–143.
14 ROSSITER, R. J., Chemical constituents of brain and nerve. In K. A. C. ELLIOTT, I. H. PAGE AND J. H. QUASTEL (Eds.), *Neurochemistry*, Thomas, Springfield, Ill., 1962, pp. 10–54.
15 SABATINI, D. D., BLOBEL, G., NONOMURA, Y., AND ADELMAN, M. R., Ribosome membrane interaction. Structural aspects and functional implications. In F. CLEMENTI AND B. CECCARELLI (Eds.), *Advances in Cytopharmacology, Vol. 1*, Raven Press, New York, 1971, pp. 119–129.
16 SELLINGER, O. Z., AZCURRA, J. M., JOHNSON, D. E., OHLSSON, W. G., AND LODIN, Z., Independence of protein synthesis and drug uptake in nerve cell bodies and glial cells isolated by a new technique, *Nature New Biol.*, 230 (1971) 253–256.
17 SELLINGER, O. Z., AZCURRA, J. M., JOHNSON, D. E., OHLSSON, W. G., KOHL, H. H., AND ZAND, R., Neurochemical correlates of drug-induced seizures: selective inhibition of cerebral protein synthesis by methionine sulfoximine, *Fed. Proc.*, 31 (1972) 160–165.
18 SELLINGER, O. Z., JOHNSON, D. E., SANTIAGO, J. C., AND IDOYAGA-VARGAS, V., A study of the biochemical differentiation of neurons and glia in the rat cerebral cortex. In D. H. FORD (Ed.), *Neurobiological Aspects of Maturation and Aging, Progress in Brain Research, Vol. 40*, Elsevier, Amsterdam, 1973, in press.
19 SELLINGER, O. Z., OHLSSON, W. G., FRANKEL, A. J., AZCURRA, J. M., AND PETIET, P. D., A study of the nascent polypeptides synthesized by the free polyribosomes of rat brain *in vivo*, *J. Neurochem.*, 18 (1971) 1243–1260.
20 SELLINGER, O. Z., OHLSSON, W. G., KOHL, H. H., AND ZAND, R., Precursor and age-dependent differences in *in vivo* rates of protein synthesis in the cerebral cortex, *Brain Research*, 34 (1971) 413–417.
21 SHAPIRO, A. L., VINUELA, E., AND MAIZEL, J., JR., Molecular weight estimation of polypeptide chains by electrophoresis in SDS-polyacrylamide gels, *Biochem. biophys. Res. Commun.*, 28 (1967) 815–819.
22 SHASHOUA, V. E., AND WOLF, M. K., CNS tissue cultures. Changes in the ratio of incorporation of leucine and valine into proteins during myelogenesis, *J. Neurochem.*, 18 (1971) 1149–1152.
23 WARECKA, K., AND BAUER, H., Studies on brain specific proteins in aqueous extracts of brain tissue, *J. Neurochem.*, 14 (1967) 783–787.
24 WARECKA, K., AND MÜLLER, D., The appearance of human 'brain-specific' glycoprotein in ontogenesis, *J. Neurol. Sci.*, 8 (1969) 329–345.
25 WEISENBERG, R. C., BORISY, G. G., AND TAYLOR, E. W., The colchicine-binding protein of mammalian brain and its relation to microtubules, *Biochemistry*, 7 (1968) 4466–4479.
26 WENDER, M., AND WALIGORA, Z., The content of amino acids in the proteins of the developing nervous system of the guinea pig. II. Cerebral grey matter, *J. Neurochem.*, 9 (1962) 115–118.
27 WILSON, L., BRYAN, J., RUBY, A., AND MAZIA, D., Precipitation of proteins by vinblastine and calcium ions, *Proc. nat. Acad. Sci. (Wash.)*, 66 (1970) 807–814.