PROTEIN SYNTHESIS IN SYNAPTOSOMAL FRACTIONS

Ultrastructural Radioautographic Study

P. GAMBETTI, L. A. AUTILIO-GAMBETTI, N. K. GONATAS, and B. SHAFER

From the Division of Neuropathology (Departments of Neurology and Pathology), University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104

ABSTRACT

A quantitative ultrastructural radioautographic study of in vitro protein synthesis has been carried out in rat synaptosomal fractions incubated with tritiated leucine or a tritiated amino acid mixture. Analysis of grain density distribution demonstrated that presynaptic endings are labeled. 30–50% of the developed grains, representing tritiated amino acids incorporated into proteins, were related to presynaptic endings which accounted for 75–77% of the total processes. 34–45% of the grains were related to processes containing ribosomes which accounted for only 4–7% of the total processes. The relative specific activity of these ribosome-containing processes, some of which could be identified as postsynaptic elements, was up to ten times higher than that of the presynaptic ending. These findings indicate that protein synthesis takes place in vitro in presynaptic terminals although to a significantly lesser degree than that occurring in ribosome-containing processes, which, with other nonpresynaptic processes, are at the present time unavoidable contaminants of synaptosomal fractions. Presynaptic endings that in radioautographs contained no mitochondria were labeled. Also, presynaptic endings were labeled after incubation in the presence of chloramphenical which inhibited 20% of the protein synthesis of the synaptosomal fraction. It is concluded that besides mitochondrial protein synthesis, another protein synthesizing system operates in presynaptic endings in vitro.

INTRODUCTION

Numerous studies have been carried out in an attempt to clarify whether protein synthesis occurs in presynaptic endings (PE).[1] Significant radioactivity related to proteins has been demonstrated in PE by ultrastructural radioautography of synaptosomal fractions after in vivo intracerebral injections of labeled amino acids (1). This could be the result of protein synthesis in the PE itself, of fast axoplasmic flow of labeled proteins from the perikaryon, or of uptake of proteins from the extracellular space and adjacent cellular structures. Biochemical studies have shown incorporation of labeled amino acids into proteins by synaptosomal fractions (2–4), but, on ultrastructural examination, the synaptosomal fractions included, in addition to PE, a significant number of nonpresynaptic processes (NP), some of which contained ribosomes (3). Since previous investigations failed to show unequivocally that protein synthesis occurs at the PE, the present ultrastruc-

[1] The following abbreviations are used in this paper: PE, presynaptic ending; NP, nonpresynaptic process; NP + R, nonpresynaptic process with ribosomes; NP − R, nonpresynaptic process without ribosomes; CAP, chloramphenicol; TCA, trichloroacetic acid; HD, half distance.

tural radioautographic study was undertaken to determine the contribution of the various components of synaptosomal fractions to protein synthesis in vitro. Using quantitative radioautography, we found that PE were indeed labeled and contained 30–50% of the radioactivity in the synaptosome fraction. Additional experiments excluded the possibility that the labeled proteins were synthesized exclusively in synaptosomal mitochondria. Preliminary reports of part of this work have been presented (5, 6).

MATERIALS AND METHODS

Materials

Ficoll was obtained from Pharmacia Fine Chemicals Inc., Piscataway, N. J.; chloramphenicol (CAP) from Sigma Chemical Co., St. Louis, Mo.; leucine-^{3}H (55.5 Ci/mmole), phenylalanine-^{3}H (8.3 Ci/mmole), alanine-^{3}H (2.93 Ci/mmole), and proline-^{3}H (5.1 Ci/mmole) from New England Nuclear Corp., Boston, Mass.

Specimen Preparation

Synaptosomal fractions were obtained on Ficoll gradients from 18-day-old Osborn-Mendel white rats as previously described (3). Incubations for amino acid incorporation were performed at 37° C in 5 ml containing 33 mM Tris-HCl pH 7.4, 0.1 M sucrose, 100 mM Na, 10 mM K, and 1 ml of synaptosomal fraction suspended in 0.32 M sucrose (approximately 5 mg protein). Three sets of experiments were carried out. (1) 50 μCi of leucine-^{3}H was added as precursor; (2) 50 μCi of each leucine-^{3}H, phenylalanine-^{3}H, alanine-^{3}H, and proline-^{3}H were used as precursors; (3) was done in parallel with (2), with the addition of CAP to a final concentration of 3×10^{-4} M. After 30 min, when incorporation ceased to be linear (3), the incubations were stopped by addition of ice cold 0.32 M sucrose containing 3 mM of the unlabeled amino acids used as precursors. The suspensions were centrifuged at 48,000 *g* for 30 min in the cold; the pellets were resuspended and washed six more times. After the final wash, each pellet was suspended in 0.32 M sucrose with a loose Dounce homogenizer to a known volume and divided into three identical portions. In one portion, the radioactivity incorporated into protein was determined as previously described (3), except that the protein, dissolved in 2 N NaOH, was counted in 15 ml of scintillation fluid (1 volume 0.01% dimethyl-POPOP (*p*-bis[2-(5-phenyloxazolyl)]benzene), 0.4% PPO(2,5-diphenyloxazole) in toluene, plus 0.5 volume of ethanol). The free radioactivity (TCA soluble) was determined by counting portions of the supernatants. The other two portions were fixed overnight in several changes of a solution containing 4% paraformaldehyde, 0.1 M cacodylate buffer (pH 7.2), and 2 mM $CaCl_2$, postfixed for 1 hr in Dalton's fixative (7), dehydrated in graded ethanol up to 90% solution and embedded directly in Epon. The entire procedure was carried out at 0–4° C. Before each step, the fractions were centrifuged at 48,000 *g* for 10 min and the radioactivity released in each supernatant was recorded. One portion, after elimination of excess Epon, was dissolved in Soluene 100 (Packard Instrument Co., Inc., Downers Grove, Ill.) and used to determine total radioactivity, while the other was allowed to polymerize at 60° C.

The radioactivity recovered during fixation and dehydration accounted for 95–105% of the TCA-soluble radioactivity present in the unfixed fraction (Table I). The total radioactivity recovered in the fixed and dehydrated pellets accounted for over 98% of the TCA-insoluble radioactivity of the unfixed fraction (Table I). Further extraction of the fixed and dehydrated fractions with 5% cold TCA removed only 1–2% of the radioactivity. 1–3% of the label was released from either fixed or unfixed fractions by subsequent treatment with ethanol:ether (3:1) and chloroform:methanol (2:1). All samples were counted in a Packard Tri Carb Scintillation Counter.

TABLE I

Recoveries of Radioactivity during Preparation for Electron Microscopy

	TCA-soluble (dpm)	Fixatives + ethanols (% recovered)	TCA insoluble (dpm)	Processed pellet (% recovered)
Experiment 1 (Leucine-^{3}H)	184,075	105	96,960	99.8
Experiment 2 (amino acid-^{3}H mixture	1,476,752	95	172,800	98.0
Experiment 3 (amino acid-^{3}H mixture + CAP)	1,362,204	100.1	130,647	99.2

The radioactivity incorporated into protein in μCi/mg protein was 1.3×10^{-2} (Experiment 1), 4.55×10^{-2} (Experiment 2), and 3.68×10^{-2} (Experiment 3).

Quench corrections were made using the channel ratio method.

Sections with light gold interference color (about 1000 A thick) (8) were cut on an LKB ultrotome and processed for ultrastructural radioautography according to the method of Salpeter (9), with minor modifications. Sections were stained for 3 hr with saturated aqueous solution of uranyl acetate followed by 10–15 min of staining with lead citrate (10), and were coated with a monolayer of Ilford L_4 emulsion, occasionally using a semiautomatic coating instrument (11); they were exposed for 8–24 wk and developed with Microdol X (Eastman Kodak Co., Rochester, N. Y.) (9).

Analysis of the Radioautograms

The sections were examined on grids with squares of 300 mesh. Since the over all grain density was low, selected electron micrographs were taken to include every developed grain seen in grid openings containing sections. The micrographs were enlarged to a final magnification of 36,000. In addition, random photographs were taken to determine the composition of the fractions. All processes that measured at least 0.3 μ in diameter and contained three or more synaptic vesicles were considered to be PE. Processes of similar size, but lacking synaptic vesicles, were considered to be NP and were classified according to the presence of (NP + R) or absence of ribosomes (NP − R) in the cytoplasm (Figs. 1 a and 1 b). Fragmented, unrecognizable processes and free mitochondria were tabulated together. The percentage of PE, NP + R, and NP − R was determined. The relative area occupied by these processes and by unidentifiable processes and fragments was calculated from the number of points (placebo points) (12) falling over these components when a lattice with points 1 cm apart was superimposed on the random photographs. Using the selected radioautographs, the geometrical center of each photographed grain was determined (13, 14), and the grains were tabulated according to the component of the fractions over which they fell. The distance from the center of each grain to the limiting membrane of the nearest process was recorded for all grains falling inside or outside the processes. All the distances were expressed in units of half distance (HD)[2] (13, 14). No relationships of grains to processes were tabulated for distances greater than 4 HD.

[2] HD is an experimental measure of resolution; it is the distance from a line source in a radioautographic specimen within which half of the developed grains fall (see References 12 and 13 for details). For the radioautograms used in this study, HD was approximately 1600 A (12).

Determination of Relative Radioactivity

The relative grain density for each component of the fraction was obtained by dividing the percentage of grains falling over that component by the percentage of area occupied by each component as determined by the random photographs. This was considered a reasonable estimate of the relative radioactivity of the different components. Because, in a preliminary counting, the relative grain density of the NP + R was much higher than that of the other components, all of the grains falling outside of these processes up to 4 HD from their limiting membrane were attributed to them due to expected net radiation spread. The relative grain densities of PE and NP − R were definitely higher than that of the fragments, and all remaining grains falling within 4 HD distance from these two processes were likewise attributed to them, even if the grains were lying over fragments. Since the relative grain densities of PE and NP − R were of the same order, those grains falling simultaneously within 4 HD of both a PE and a NP − R were divided equally between the two processes. The background density, determined for each grid using grid squares that did not contain sections, was 5–10% of the grain density found over the sections. Corrections were then made for each component of the fraction according to the per cent of area occupied as determined by the random photographs. Grains were considered *related* to the PE, NP + R, or NP − R if, after correction for radiation spread and background, they fell over or outside of these processes up to a distance of 4 HD. The remaining grains were attributed to fragments unless they fell more than 4 HD from any structure, in which case they were ignored.

Histograms of grain density distribution were made according to Salpeter and coworkers (12, 13), except that the "cross-scattered" radiation of the PE was not corrected (see Reference 13 for details). Briefly, Salpeter et al. (12) demonstrated that when grain density distributions are tabulated in distance units of HD from a given source, these distributions have a universal shape that is independent of resolution. In the present study, grain density[3] distributions over and around PE and NP + R were tabulated in distance units of HD inward and outward from the limiting membrane of these processes. The grain density was normalized at the limiting membrane (HD = O). To determine whether PE and NP + R were indeed labeled, the normalized histograms were compared with the theoretical distribution expected from radioactive sources similar in size and shape.

[3] The grain density of a given compartment is obtained by dividing the number of grains by the number of "placebo points" falling within the same compartments.

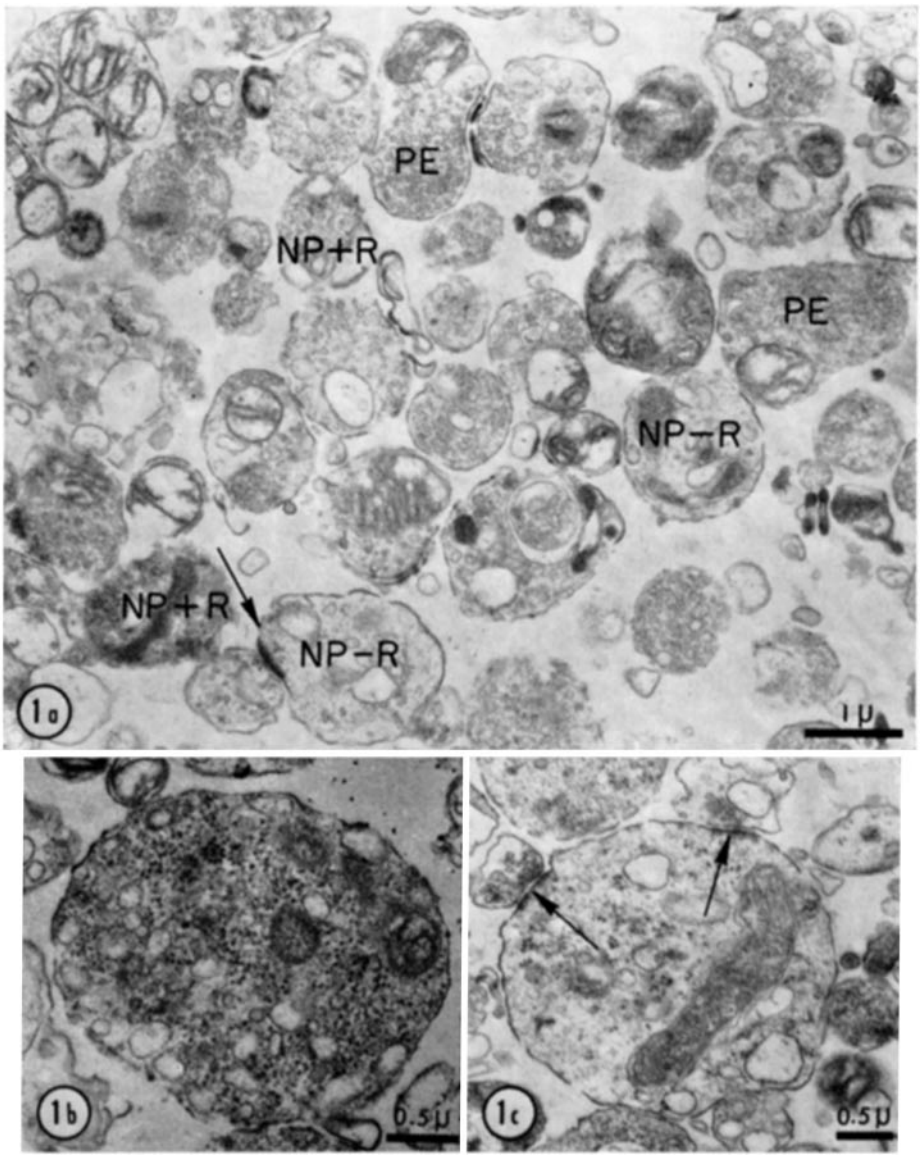

FIGURE 1 Fig. 1 a, Synaptosomal fraction from 18 day old rat cerebral cortex. Most of the processes are presynaptic endings (*PE*). Some processes lack synaptic vesicles and ribosomes (*NP − R*); one of them is a postsynaptic element (arrow). Two processes contain ribosomes (*NP + R*). × 13,500. Fig. 1 b, Detail of ribosome-containing process. × 20,000. Fig. 1 c, Ribosome-containing process forming synaptic contacts with two PE (arrows). × 15,000.

Histograms for PE were obtained from each of the three experiments. In experiment 2, histograms were plotted separately for PE containing and lacking mitochondria; in addition, a histogram was plotted for the NP + R.

RESULTS

Composition of the Fractions

PE accounted for 70–75% of the identifiable processes (Fig. 1 a, Table II). (NP−R) accounted

TABLE II

Distribution of Grains among the Various Components of the Fractions

	Grain distribution (% of total)			Fraction composition (% of processes)*			Area occupied by fraction (% components)‡			RSA $\frac{(\%\ \text{Grains})}{(\%\ \text{area})}$		
	(1)	(2)	(3)	(1)	(2)	(3)	(1)	(2)	(3)	(1)	(2)	(3)
Presynaptic	31.5	52.6	42.1	77.3	75.2	70.4	19.3	25.5	24.4	1.6	2.1	1.7
NP + R	46.7	33.8	39.2	7.4	4.6	3.3	2.9	2.6	1.6	16.1	13.0	24.5
NP − R	8.4	9.0	13.3	15.3	20.2	26.3	4.4	7.1	8.0	1.9	1.3	1.7
Fragments	13.3	4.6	5.6				73.5	64.6	65.5	0.18	0.07	0.08

(1) Synaptosomal fractions were incubated for protein synthesis with leucine-^{3}H as precursor. (2) amino acid-^{3}H mixture was used as precursor. (3) The same as (2) with the addition of 3×10^{-4} M chloramphenicol. The grain distributions were based on 428 (1), 577 (2), and 505 grains (3).

* Based on 2098 processes counted in (1), 2,109 in (2) and 2,120 in (3).

‡ Calculated from random pictures. The areas surveyed were 950 μ^2 in (1), 1900 μ^2 in (2), and 1520 μ^2 in (3).

for 15–26% (Fig. 1 a), while (NP+R) (Figs. 1 a–c) accounted for 4–7% (Table II). PE accounted for the greatest area occupied by these processes (Table II).

Radioautographic Study of Experiment 1 (Leucine-^{3}H as Precursor) and Experiment 2 (AA-^{3}H Mixture as Precursor)

Visual scanning of developed radioautograms showed that the grains were frequently concentrated over NP+R while more than one grain over a PE was rarely seen (Figs. 2 a, b). After several hundred grains were tabulated, 31–53% of the grains were found to be related to the PE in experiments 1 and 2 (Table II). 34–47% of the grains were related to the NP+R (Table II). The relative specific activity (RSA) of the PE was ten times less than that of the NP+R in experiment 1 and six times less than that in experiment 2 (Table II).

Histograms of the grain density distribution were similar for the PE in both experiments (Figs. 3 a, b). The grain density *outside* the PE, particularly in experiment 1, was higher than the theoretical curve. This finding was expected since occasionally two or more PE were closer than 4 HD to each other, and the surrounding area they shared had a higher grain density; no correction could be made for this "cross-scattered radiation" (13). Nevertheless, both histograms show that the grain densities inside the PE are much higher than outside, where they progressively diminish at increasing distances from the PE. Therefore, it can be concluded that PE are indeed labeled in both experiments. The grain density inside the PE in both histograms was lower than theoretically expected from a uniformly labeled source (Figs. 3 a, b). This suggests that the radioactivity is not uniformly distributed, but is relatively higher at the periphery. According to Salpeter et al. (12, 13), one can obtain curves which fit the experimental data best by combining, in different ratios, the theoretical curve obtained when the radioactive source is a uniformly labeled solid disc and that obtained when the source is shaped as a hollow circle. Following this method (12, 13), it was determined that the curve which best fits the present data is that expected if about 60% of the radioactivity is uniformly distributed throughout the PE and the remaining 40% concentrated only at the periphery. However, because of the large standard deviation due to the low number of grains and points plotted for the "inside" portion of the histogram, these figures are questionable (Figs. 3 a, b). The histogram for the NP+R of experiment 2 fitted the curve of the theoretical distribution expected from a uniformly labeled source of similar size and shape.

Radioautographic Study of the Effect of Chloramphenicol (CAP) (Experiment 3)

The addition of CAP inhibited amino acid incorporation by 20% (Table I). In this fraction, 42% of the grains were related to the PE. As compared to the control (experiment 2), this represents a 20% diminution (Table II). The relative specific activity was 1.7, also lower than

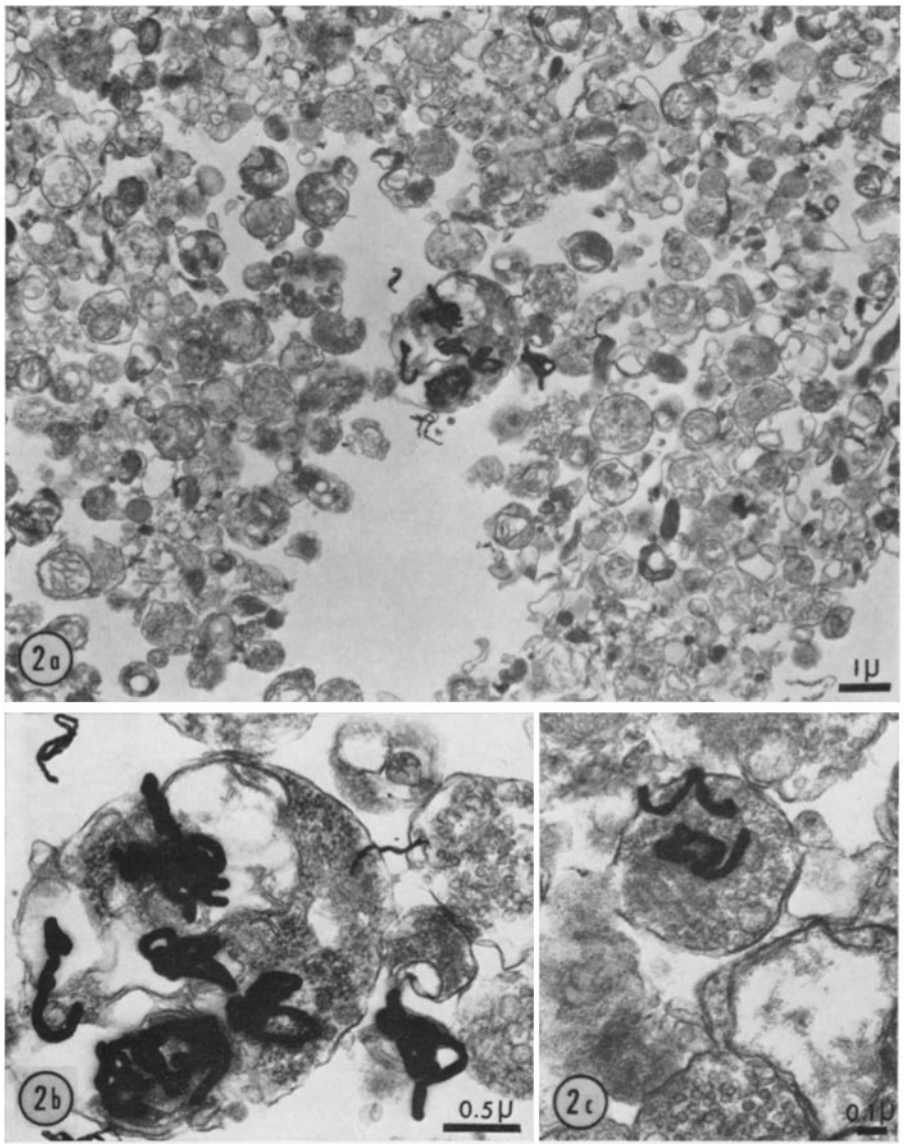

FIGURE 2 Fig. 2 a, Radioautogram from experiment 2. Most of the developed grains are concentrated in a ribosome-containing process. × 7000. Fig. 2 b, Detail, × 20,000. Fig. 2 c, PE with two grains. This was a rare finding; the grains over PE were generally no more than one. × 38,000.

in the control (2.1). In the histogram of grain density distribution (Fig. 4), the grain densities outside the PE were similar to experiments 1 and 2 (Figs. 3 a, b) and demonstrate that the PE are labeled. By contrast, the distributions inside the PE were quite different. The theoretical curve fitting the experimental data was that for a source having the shape of a hollow circle (12). The null hypothesis that the "inside" portions of this histogram (Fig. 4) and that of the histogram from experiment 2 (Fig. 3 b) came from the same distribution, whose true shape is the average of the two, was tested using χ^2. The probability that each curve has this large a deviation in the expected direction purely by chance was found to be $<1\%$. This indicates that in this experiment

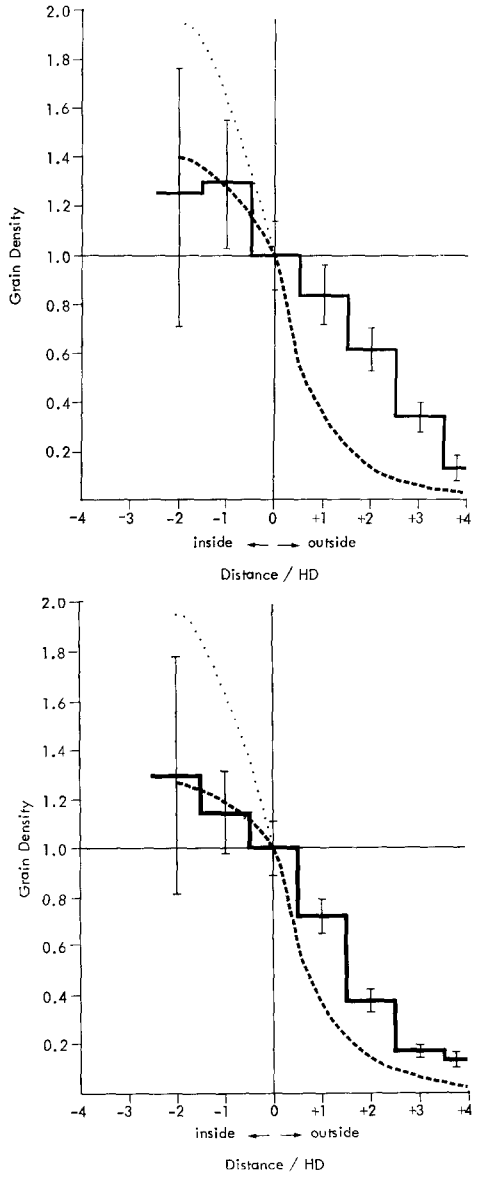

FIGURE 3 Fig. 3 a, Histogram of distribution of grain densities over and around PE. Leucine-^{3}H was used as precursor. (Based on 230 grains). Fig. 3 b. Histogram of distribution of grain densities over and around PE. Amino acids-^{3}H mixture was used as precursor. (Based on 383 grains.) ....., Theoretical curve of grain density distribution expected for a uniformly labeled radioactive source equal in size and shape to the average PE. ------, Theoretical curve fitting the experimental histograms; this corresponds to the curve expected if about 60% of the radioactivity is uniformly distributed throughout the PE and the remaining 40% is concentrated at the periphery. The

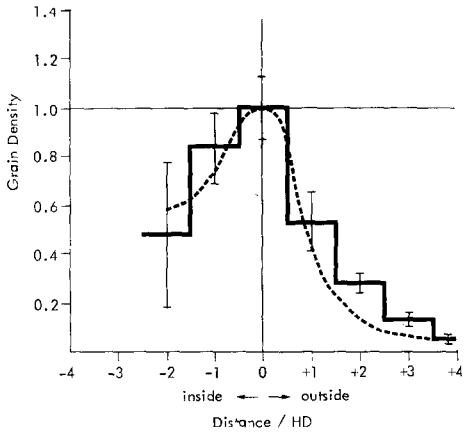

FIGURE 4 Histogram of distribution of grain densities over and around PE incubated for protein synthesis with amino acid-^{3}H mixture in the presence of 3×10^{-4} M chloramphenicol. The superimposed dashed curve fitting the histogram corresponds to the distribution expected for a hollow circle equal in size to the average PE (Based on 290 grains).

the central region of the PE has little or no radioactivity.

Additional Radioautographic Studies

To further substantiate the conclusion that mitochondria were not the only site of synthesis of labeled protein in the PE, a histogram of grain density distributions was obtained for PE that contained no mitochondria in the radioautograms (Fig. 5). Again, the grain densities outside the process diminished progressively at increasing distances from the limiting membrane (Fig. 5). This clearly shows that the PE not containing mitochondria are also labeled.

DISCUSSION

The presence of processes other than PE, some containing ribosomes, has been observed previously in rat and mouse synaptosomal fractions prepared in sucrose or Ficoll gradients (3, 15–18). In fractions from 18-day-old rats, NP consistently account for 20–30% of the identifiable processes (17).

formula $G/P \sqrt{(1/\sqrt{G})^2 + (1/\sqrt{P})^2}$, where G equals the number of grains and P equals the number of points, multiplied by the same factor used to convert grain density over the limiting membrane to unity (15), was used for determining ± standard deviations in all of the histograms.

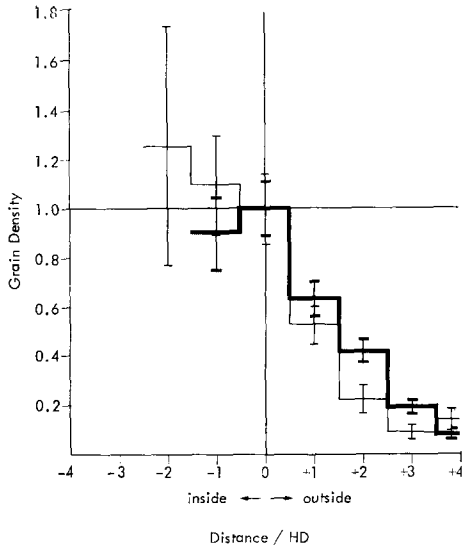

FIGURE 5 Superimposed histograms of distribution of grain densities over and around PE which in the radioautograms contained or lacked mitochondria. ——, PE lacking mitochondria (based on 380 grains). ——, PE containing mitochondria (based on 168 grains).

This figure is of the same order as those found in the present study, indicating that the incubation for amino acid incorporation and the washes before fixation did not change the relative composition of the fractions or affect the recognition of the various components. NP are less numerous in fractions from adult rats but they still acount for up to 13% of the total (17). The origin of the NP is difficult to assess. It has been suggested that the NP containing ribosomes (NP+R) come from oligodendrocytes (15). At least some of the NP+R and NP−R are postsynaptic elements (Figs. 1 a, c). It is likely that the NP−R are distal portions of dendrites or dendritic spines, while the NP+R could be either proximal portions of dendrites (frequently endowed with numerous ribosomes) (19) or portions of neuronal perikaryon pinched off at the site of axo-somatic synapses.

The present study demonstrates that after incubation of synaptosomal fractions for protein synthesis, the PE are labeled and contain up to 50% of the radioactivity. Special care was taken to ascertain that all the radioactivity remaining in the fraction after fixation and dehydration was incorporated into proteins. Evidence for the lack of free precursor or TCA-insoluble products other than protein was provided by the negligible amounts of radioactivity removed from the processed fractions by 5% TCA and by subsequent extractions with lipid solvents.

Since PE contain no polysomes or rough endoplasmic reticulum, which are the conventional sites of cytoplasmic protein synthesis, the origin of newly synthesized synaptosomal protein remained unclear. We attempted to investigate the possibility that all labeled proteins found in synaptosomes were synthesized in synaptosomal mitochondria. CAP was used because of considerable evidence that it inhibits mitochondrial protein synthesis in brain (16, 20–23). PE from synaptosomal fractions incubated for protein synthesis in the presence of CAP were labeled (Fig. 4). The radioactivity inside the PE had a different distribution and appeared to be confined to the region of the limiting membrane, while in the control, the central area of the PE was also labeled (Fig. 3 b). The significance of this finding, however, is limited by the lack of a direct proof of a complete inhibition of mitochondrial protein synthesis in PE.

Therefore, a second approach was used. Grain density distribution of PE lacking mitochondria in the radioautograms was calculated separately from that of PE with mitochondria. PE that lacked mitochondria were labeled (Fig. 5). Since there is no evidence that proteins synthesized in mitochondria are transported into the cytoplasm (24), the finding of label in PE not containing mitochondria strongly suggests that protein synthesis in PE takes place also outside mitochondria. The possibility was considered that the labeled nonmitochondrial proteins in the PE were synthesized by other components of the synaptosomal fraction and that they were either taken up by the PE or that they simply adhered to its limiting membrane. This possibility, however, is unlikely since we found no free proteins in the medium after incubation of the synaptosomal fractions. Furthermore, other experiments in our laboratory (to be reported shortly) showed no protein uptake from the medium, when synaptosomal fractions were incubated with labeled soluble proteins obtained after osmotic shock of a previously labeled synaptosomal fraction. The present study therefore indicates that PE are capable of synthesizing proteins in vitro and that synaptosomal mitochondria contribute to, but are not the only site of, this synthesis. Assuming equal amino acid pools, protein synthesis taking place in the presynaptic

endings is up to ten times less active than that of the processes containing ribosomes.

Finally, the present findings also indicate that caution should be used in attributing exclusively to the PE metabolic features observed in "synaptosomal fractions," since other elements, although small in amount, may contribute significantly to the metabolism of the fraction.

When this manuscript was already completed, Cotman and Taylor (25) reported a radioautographic study of synaptosomal fractions which showed that 48% of the radioactivity was related to presynaptic endings; morphological composition of their synaptosomal fractions, and density of silver grains related to the presynaptic endings and other processes were not investigated; furthermore, no definite proof was provided that observed grains indeed represented labeled proteins

We are deeply grateful to Dr. Miriam Salpeter for her continuous valuable advice and support. The technical assistance of Miss Lieselotte Hirt and Miss Anna Stieber is appreciated.

This paper was supported by grants NS 08933-01 and NS 05572-07.

Received for publication 12 August 1971, and in revised form 26 October 1971.

REFERENCES

1. Droz, B., and S. H. Barondes. 1969. Nerve endings: rapid appearance of labeled protein shown by electron microscope radioautography. *Science (Washington).* **165:**1131.
2. Morgan, I. G., and L. Austin. 1968. Synaptosomal protein synthesis in a cell-free system. *J. Neurochem.* **15:**41.
3. Autilio, L. A., S. H. Appel, P. Pettis, and P. Gambetti. 1968. Biochemical studies of synapses in vitro. I. Protein synthesis. *Biochemistry.* **7:**2615.
4. Bosmann, H. B., and B. A. Hemsworth. 1970. Intraneural mitochondria. *J. Biol. Chem.* **245:** 363.
5. Gambetti, P., L. Autilio-Gambetti, and N. K. Gonatas. 1970. Ultrastructural radioautographic study of protein synthesis in synaptosomal fractions. *J. Cell Biol.* **47:**68 a. (Abstr.)
6. Autilio-Gambetti, L., P. Gambetti, and N. K. Gonatas. 1971. Protein synthesis in synaptosomal fractions. Autoradiographic study. *Fed. Proc.* **30:**1139.
7. Dalton, A. H. 1955. A chrome-osmium fixation for electron microscopy. *Anat. Rec.* **121:**281.
8. Faeder, I. R., and M. M. Salpeter. 1970. Glutamate uptake by a stimulated insect nerve muscle preparation. *J. Cell Biol.* **46:**300.
9. Salpeter, M. M. 1966. General area of autoradiography at the electron microscope level. *In* Methods in Cell Physiology. D. M. Prescott, editor. Academic Press Inc., New York. 229.
10. Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* **17:**208.
11. Kopriwa, B. M. 1967. A semiautomatic instrument for the radioautographic coating technique. *J. Histochem. Cytochem.* **14:**923.
12. Salpeter, M. M. 1968. H^3-Proline incorporation into cartilage: Electron microscope autoradiographic observations. *J. Morphol.* **124:**387.
13. Salpeter, M. M., L. Bachman, and E. E. Salpeter. 1969. Resolution in electron microscope radioautography. *J. Cell Biol.* **41:**1.
14. Budd, G. C., and M. M. Salpeter. 1969. The distribution of labeled norepinephrine within sympathetic nerve terminals studied with electron microscope radioautography. *J. Cell Biol.* **41:**21.
15. Hedley-Whyte, E. T., F. A. Rawlins, M. M. Salpeter, and B. G. Uzman. 1969. Distribution of cholesterol-1,2-H^3 during maturation of mouse peripheral nerves. *Lab. Invest.* **21:**536.
16. Johnston, N. L., and L. M. H. Larramendi. 1968. The separation and identification of fractions of non-myelinated axons from the cerebellum of the cat. *Exp. Brain Res.* **5:**326.
17. Morgan, I. G. 1970. Protein synthesis in brain mitochondrial and synaptosomal preparations. *Fed. Eur. Biochem. Soc. Letters.* **10:**273.
18. Gonatas, N. K., L. A. Autilio-Gambetti, P. Gambetti, and B. Shafer. 1971. Morphological and biochemical changes in rat synaptosome fractions during neonatal development. *J. Cell Biol.* **51:**484.
19. Flexner, L. B., P. Gambetti, J. B. Flexner, and R. B. Roberts. 1971. Studies on memory. Distribution of peptidyl-puromycin in subcellular fractions of mouse brain. *Proc. Nat. Acad. Sci. U. S. A.* **68:**26.
20. Peters, A., S. L. Palay, and H. de F. Webster. 1970. The fine structure of the nervous system. The cells and their processes. Harper & Row, Publishers, New York. 48.
21. Mahler, H. R., L. R. Jones, and W. J. Moorf. 1971. Mitochondrial contribution to protein synthesis in cerebral cortex. *Biochem. Biophys. Res. Commun.* **42:**384.
22. Haldar, D. 1971. Protein synthesis in isolated rat brain mitochondria. *Biochem. Biophys. Res. Commun.* **42:**899.

23. AUSTIN, L., I. G. MORGAN, and J. J. BRAY. 1970. The biosynthesis of proteins within axons and synaptosomes. *In* Protein Metabolism of the Nervous System. A. Lajtha, editor. Plenum Publishing Corporation, New York. 271.
24. HENSON, C. P., C. N. WEBER, and H. R. MAHLER. 1968. Formation of yeast mitochondria. I. Kinetics of amino acid incorporation during depression. *Biochemistry*. **7**:4431.
25. COTMAN, C. W., and D. A. TAYLOR. 1971. Autoradiographic analysis of protein synthesis in synaptosomal fractions. *Brain Res.* **29**:366.