Rapid Appearance of Labeled Lecithin and Protein in Isolated Nerve Endings from Rat Brain[1] (34897)

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Since the hypothesis advanced by Weiss and Hiscoe (1) that part of the synaptic components originates in the cell body of the neuron and flows down the axon, a considerable amount of evidence has accumulated in the past few years showing that amino acids, when injected directly into the brain, are preferentially taken up into the cell body (2, 3). The synthesized proteins are transported then toward the synaptic endings by the slow process of somatoaxonal flow.

Studies from this laboratory on the subcellular distribution of acetylcholine, acetylcholinesterase and sodium–potassium adenosine triphosphatase (Na^+–K^+–ATPase) during brain growth and development suggested a functional relationship between the microsomes in the cell body and the synaptic endings (4).

To throw more light on the relationship between the metabolism of the cell body of the neuron and its nerve terminal the *in vivo* incorporation of ^{14}C-leucine into proteins and ^{14}C-choline into lecithin of the microsomes and nerve endings of rat brain were investigated in the presence and absence of puromycin and the properties of Na^+–K^+–ATPase in both particulates were compared.

Materials and Methods. Administration of Radioactive Precursors and Subcellular Fractionation. Rats at ages 12–18 days were injected intracranially. The amount of precursor administered per g of brain was 5 μCi (15 mCi/mmole) of choline-methyl-^{14}C chloride, and 4 μCi (250 mCi/mmole) L-leucine ^{14}C (U). A group of three rats from the same litter was used for each precursor and time of experiment. Puromycin (0.1 mg adjusted to pH 7.2 with NaOH) was injected simultaneously with the radioisotope as indicated. After injection, the young rats were returned to their motheers until required. At the end of experiment, the rats were sacrificed, their cerebra removed, then combined for each group and homogenized in 0.25 *M* sucrose. The nerve endings were obtained by subfractionating the crude mitochondrial fraction by means of discontinuous density-gradient centrifugation as described previously (5). The microsomal fraction was obtained by first centrifuging the postmitochondrial supernatant fluid at 15,000 rpm for 20 min to obtain a precipitate and supernate. The latter was centrifuged at 40,000 rpm for 1 hr to give a fairly purified microsomal preparation when examined with the electron microscope.

Extraction and analysis of lipids. The precipitates obtained from the lysed preparations after centrifugation at 50,000 rpm for 30 min were homogenized in 8 ml of chloroform–methanol–HCl (200:100:1) containing 0.05 g α-tocopherol per 100 ml extractant, then placed in a water-bath shaker at 50° for 15 min and left overnight at 0°. The extracts were centrifuged at 4000 rpm for 30 min, and the supernatant fluids were extracted twice with 0.2 vol saline. The organic layer was filtered, concentrated *in vacuo*, and the lipid concentrate taken up in 0.5 ml chloroform. Lecithin was isolated from the lipid extract on silica Gel G by means of two-dimensional TLC and its radioactive specific activity (sp act) was determined as described previously (6).

For the *in vitro* incorporation of ^{14}C-choline into lecithin, incubations were carried out in Erlenmyer flasks (25-ml capacity) for 2 hr with shaking in a water-bath shaker at 37°. Nerve endings or microsomes, suspended

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in 1 ml of 0.45 *M* sucrose and corresponding to 12–20 mg protein, were added to an incubation medium which consisted of 20 m*M* pyruvate, 1.22 m*M* malate, 3.3 m*M* glucose, 1.67 m*M* α-glycerol phosphate, 5 m*M* choline chloride, 33.3 m*M* K-phosphate buffer, pH 7.4, 5 m*M* $MgCl_2$, 2 m*M* ATP, 1 m*M* CTP, 0.51 m*M* CoA, 3.73 m*M* NAD, and 0.1 ml saline containing 3 μCi of ^{14}C-choline in a final volume of 3.0 ml. Puromycin (0.1 mg) was added as indicated. The phospholipids were analyzed as described above.

Extraction and analysis of ^{14}C-leucine-labeled proteins. The subcellular fractions were homogenized in deionized water, and the lysed precipitate was then homogenized in 5% TCA containing 10 m*M* L-leucine. The insoluble proteins were washed, hydrolyzed in 1 *N* NaOH and their specific activity determined according to the procedure of Sjöstrand (7).

Assay of sodium–potassium-activated ATPase. Na^+–K^+–ATPase was assayed as described previously (8).

Choline-methyl-^{14}C-chloride (15 mCi/mmole) and L-leucine-^{14}C (U) was purchased from New England Nuclear. Ficoll was purchased from Pharmacia Fine Chemicals Inc. and purified by dialysis against deionized water.

Results. Within 30 min after injection of the radioactive precursors, lecithin and proteins of the nerve endings were labeled and the sp act ratio of both were higher in the microsomal than the nerve-ending fraction (Figs. 1 and 2). The sp act of lecithin was highest in the microsomal fraction after 1 hr from time of injection, while that of the nerve endings reached its peak after 3 hr (Fig. 1). In contrast 30 min after injection of ^{14}C-leucine, the sp act of the proteins decreased steadily in the microsomal fraction but increased slightly in the nerve endings and then leveled off during the first 3 hr (Fig. 2). It can be observed that in the 3-hr experiment puromycin exerted 14% and 18% inhibition on lecithin and protein synthesis, respectively, in the microsomes, while in the

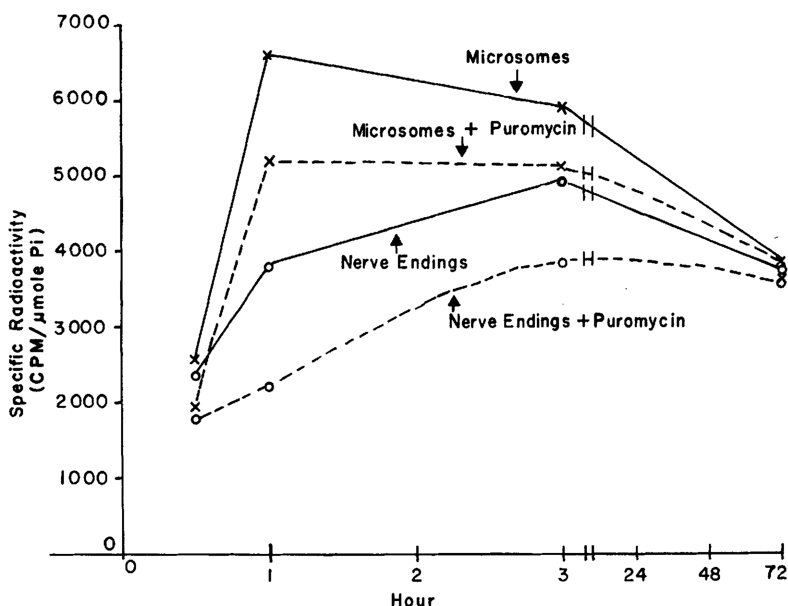

FIG. 1. The specific radioactivity of lecithin at various time intervals in the microsomal and nerve-ending fractions, expressed as cpm/μmole Pi, after intracranial injection of ^{14}C-choline in the presence and absence of puromycin. Lecithin was isolated from the lipid extract by means of two-dimensional TLC and its specific radioactivity determined. Each point on the curve represents two replicate experiments, and each experiment was analyzed in duplicates. The values of the replicate experiments agreed within 3%.

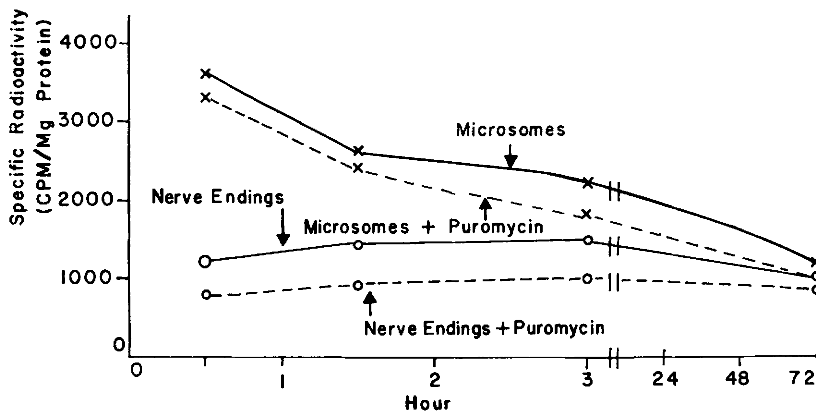

FIG. 2. The specific radioactivity of insoluble proteins at various time intervals in the microsomal and nerve-ending fractions, expressed as cpm/mg protein, after intracranial injection of ^{14}C-leucine in the presence and absence of puromycin. The insoluble proteins were washed and extracted with trichloroacetic acid, then ether-ethanol (4:1) and the precipitate hydrolyzed in 1 *N* NaOH and its specific radioactivity determined. Each point on the curve represents two replicate experiments and each experiment was analyzed in duplicates. The values of the replicate experiments agreed within 4%.

nerve-ending fraction its inhibitory effect on the former was 21% and on the latter 33% of that of the control. This inhibitory effect decreased appreciably in the 3-day experiment. The slight increase in the sp act of lipids and proteins of the nerve endings accompanied by a decrease in the sp act of those of the microsomes followed by equilibration of the sp act of both fractions in the 72-hr experiment could suggest that part of these membrane components originates in the nerve cell body and flows down the axon. Studies on a typical membraneous protein, namely Na^+–K^+–ATPase, in both particulates revealed no distinct differences in the properties of the enzyme from both particulates, although its activity in the nerve-ending fraction was considerably higher than that of the microsomes (Table I). In contrast the activity of acetyl cholinesterase was found to be higher in the microsomes than in the nerve endings (4).

Austin and Morgan (9, 10) and Autilio *et al.* (11) showed that nerve endings can incorporate amino acids into protein *in vitro*. As can be seen from Table II, nerve endings can

TABLE I. Comparative Studies on the Hydrolysis of ATP in the Presence of Nerve Endings and Microsomes of Rat Brain.

	Nerve endings		Microsomes	
	Mg^{2+}-ATPase	Na^+-K^+-Mg^{2+}-ATPase	Mg^{2+}-ATPase	Na^+-K^+-Mg^{2+}-ATPase
K_m[a] (M × 10^{-4})	0.193	2.0	0.63	2.4
V_{max}[a] (μmoles P_i liberated/hr/mg protein	23.9	50.0	21.7	33.0
K_h[b] (M × 10^{-4})	0.85	1.95	0.93	1.73
N[b]	1.13	1.09	1.15	1.10
pH optimum	8.0	8.0	8.0	8.0
Temp. optimum	37°–40°	37°–40°	37°–40°	37°–40°

[a] Obtained by plotting the data in terms of the lineweaver-Burk equation.
[b] Obtained by plotting the data in terms of the Hill equation.

TABLE II. *In Vitro* Incorporation of ^{14}C-Choline Into Lecithin of Nerve Endings and Microsomes of Rat Brain.[a]

	Specific radioactivity (cpm/μmole P_i)		
Subfraction	Control	Puromycin added	Inhibition as % of control
Nerve endings	1995	1923	3.5
Microsomes	495	480	3

[a] Reaction mixtures and conditions were those as described under Methods.

also incorporate ^{14}C-choline into lecithin *in vitro*, unexpectedly at a higher rate than that of the microsomes, and puromycin had a negligible inhibitory effect on the course of this incorporation.

Discussion. The present work on the *in vivo* incorporation of ^{14}C-choline into lecithin and ^{14}C-leucine into protein of microsomes and nerve endings is in accord with the view that in the central nervous system at least part of the phospholipids and proteins of the synapse originates in the cell body of the neuron.

In this connection, Droz and Barondes working with mouse brain showed that 90 min after intracerebral injection of tritiated leucine and lysine, radioactive protein was associated with the nerve-ending fraction (12). Sjöstrand (7) demonstrated a rapid proximo-distal transport of labeled proteins in the vagus and hypoglossal nerves after intramedullary injection of ^{3}H-leucine. Furthermore axonal transport of phospholipids which move rapidly in a somatofugal direction has been shown by Miani in the rabbit hypoglossal and vagus nerves (13). The findings that both of the sp act of proteins and lipids increase in the nerve endings with time coupled with a decrease in their sp act in the microsomes then equilibration, could be due to the migration of labeled protein and lipid synthesized in the cell perikaryon to the nerve endings. Thus, the turnover of lipids and proteins will be higher in the microsomal than in the nerve-ending fraction. On the other hand, the present finding that nerve-endings can synthesize their own phospholipids and those of others on the synthesis of proteins *in vitro* (9–11, 14) indicate to us that the metabolism of the synapse can be independent of that of the cell body of the neuron. The fact that puromycin, a potent inhibitor of protein synthesis in mammalian tissue exerted an inhibitory effect on lipid synthesis suggest a close relationship between the incorporation of ^{14}C-choline and ^{14}C-leucine into lecithin and proteins, respectively.

Although the present study does not show conclusively the transport of phospholipids and proteins from the perikaryon to the nerve terminal, the evidence presented is consistent with the view that the synapse receives its proteins and phospholipids from the cell perikaryon as well as from local synthesis. Further studies on the metabolic relationship between the microsomes, which are part of the cell body of the neuron, and nerve endings could throw more light on axoplasmic transport in the central nervous system.

Summary. Rats at ages 12–18 days were injected intracranially with ^{14}C-choline and ^{14}C-leucine in the presence and absence of puromycin, then sacrificed at various time intervals, and nerve endings and microsomes were isolated from the cerebra by means of differential and density-gradient centrifugation. The specific radioactivities of lecithin and insoluble proteins increased in nerve endings and decreased in microsomes in the short-term experiments and bcame similar from both particulates after 72 hr from time of administration. Purmoycin exerted an appreciable inhibitory effect on the synthesis of both lipids and proteins from both fractions *in vivo*, but had a negligible effect on lecithin synthesis *in vitro*. No differences were found between the properties of Na^{+}–K^{+}–ATPase from the nerve-ending and microsomal fractions. From the relationship between the synthesis of lecithin and proteins *in vivo* in both particulates and the synthesis of lecithin *in vitro*, it can be concluded that the synapse can derive its lipids and proteins from the cell body as well as from local synthesis.

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