PROPERTIES DISTINGUISHING MITOCHONDRIAL AND SYNAPTOSOMAL PROTEIN SYNTHESIS *

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SUMMARY

Mouse brain synaptosomal protein synthesis in vitro is inhibited by chloramphenicol, cycloheximide, emetine, and chloroquine,whereas mouse brain mitochondria are sensitive to inhibition only by chloramphenicol. These results, and others cited, confirm that: (1) brain mitochondria do not differ from other mitochondria except for their capacity for a faster rate of in vitro protein synthesis; (2) isolated synaptosomes apparently possess two protein synthetic systems. Furthermore, based upon the effects of combinations of inhibitors, the two systems are in some manner interdependent

INTRODUCTION

Much of the protein of the axon and presynaptic terminal in brain is supplied via axoplasmic transport from its site of synthesis in the neuron cell body (1). Nevertheless, the capacity for local synthesis might be of great importance in events at the synapse, including neurotransmission, the phenomenon of tolerance to addicting drugs, and memory storage. For these reasons the unresolved question of the nature of local protein synthesis in the synaptic region is of interest.

It is generally held that typical cytoplasmic ribosomes do not extend peripherally into the nerve cell axon beyond the axon hillock (2). But the axon and presynaptic region do contain RNA (3,4), and it is possible that

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this RNA might function in translation. Indeed, the isolated synaptic region (synaptosome) has been studied in vitro, and some capacity for protein synthesis has been reported by several workers (5-10). On the basis of studies with inhibitors of protein synthesis, which,in most reports, showed that a portion of synaptosomal protein synthesis was sensitive to chloramphenicol and that a portion was inhibited by cycloheximide, it was generally assumed that two systems, a mitochondrial and a "nonmitochondrial", were present.

This straightforward assumption could not be accepted as fact until some other confusing observations were taken into account. Early reports on brain mitochondrial protein synthesis stated that brain mitochondria differed from all others studied, in that they appeared to be sensitive to cycloheximide. Thus, if this were indeed the case, perhaps all of the synaptosomal synthesis could have been attributed to the intrasynaptosomal mitochondria (11). However, the more recent studies, utilizing improved techniques, have resolved much of the confusion. Mahler, Jones, and Moore (12) found that synthesis of soluble protein in brain slices was completely inhibited by emetine, a potent inhibitor of cytoribosomal protein synthesis (13,14), whereas a small portion of the insoluble protein synthesized was not, and that this portion, which was sensitive to chloramphenicol, could be attributed to mitochondria when the incubated slices were homogenized and subfractionated. Furthermore, the studies of Bosmann and Hemsworth (15), and those reported here, have shown that isolated brain mitochondria are, in fact, similar to other mitochondria except for a very much faster rate of synthesis; they are quite sensitive to chloramphenicol and insensitive to emetine or to cycloheximide (except at very high concentrations).

In contrast, we find that purified synaptosome preparations are sensitive not only to chloramphenicol and to cycloheximide, but also to emetine and chloroquine,permitting a clear distinction between brain mitochondria and synaptosomes.

Most investigators of in vitro protein synthesis by synaptosomal preparations have been in agreement that the system is: (1) insensitive to ribonuclease, and therefore probably bounded by a membrane; (2) inactivated by osmotic lysis (which is not expected of typical cytoplasmic ribosomes); and (3) inhibited when oxidative phosphorylation was blocked, suggesting the possibility of a coupling of the intrasynaptosomal mitochondrial and non-mitochondrial systems (16). If it is true, as seems likely (17), that chloramphenicol is specific for inhibition of mitoribosomal mediated protein synthesis and that emetine and cycloheximide are specific for inhibition of cytoribosomal mediated protein synthesis, our studies reported here are strong support for some type of coupling of the synthesis systems within the synaptosome.

MATERIALS AND METHODS

All equipment and glassware were autoclaved prior to use. All reagents were obtained from commercial sources, utilizing only those of the highest available purity. All solutions, which are prepared in deionized, glass distilled water, were passed through a 0.45μ millipore filter.

Homogenates of 9 to 12 day old Swiss mouse brains were prepared by a modification of the method previously described (18). After decapitation the brains were removed as quickly and aseptically as possible, and were homogenized at 0° by hand in all glass homogenizers. Approximately 3 grams of tissue (14 to 16 mice) were dispersed in 100 ml. of 0.33 M sucrose. It is important to note that satisfactory separation of mitochondria and synaptosomes was dependent upon the combination of gentle homogenization in large volumes of isotonic sucrose.

Nuclear debris was removed by centrifugation at 1085 x g for 10 minutes at 0° in a Servall preparative centrifuge. The supernatant fluid was centrifuged at 10,000 x g for 15 minutes, and the residue was resuspended in 100 ml. of 0.33 M sucrose. The centrifugations were repeated five times, each time decanting completely the supernatant fluid, which was discarded.

The final pellet was resuspended gently in 3 ml of 0.33 M sucrose. Aliquots of 0.5 ml. were layered onto discontinuous 4 ml. sucrose gradients made up of layers of 1 ml. each of 1.6 M, 1.4 M, 1.2 M, and 1.0 M sucrose. The gradients were centrifuged at 34,000 RPM for 80 minutes in an SW 50.1 Rotor in the Spinco L-2B centrifuge. Mitochondria were harvested at the 1.6 M - 1.4 M interface, and synaptosomes were harvested from the 1.2 M - 1.0 M interface. (Abundant documentation as to the identities of these two fractions has appeared in the literature; see, for example, (19,20).) These were diluted to a final concentration of 0.33 M sucrose with water, and sedimented by centrifugation at 10,000 x g for 15 minutes.

Incubations, in the media described in the table, were terminated by addition of an equal volume of ice cold 10 per cent trichloroacetic acid which was 10mM with respect to nonradioactive leucine. Preparation of protein precipitates and assessment of radioactivity were as described (18).

RESULTS AND DISCUSSION

During the course of experiments on characterization of protein synthesis in crude brain mitochondrial preparations, we observed that repeated resuspension of post-nuclear residue (predominantly mitochondria and synaptosomes) in large volumes of isotonic sucrose followed by centrifugation at low speeds (5,000 x g) yielded preparations which were more sensitive to inhibition by chloramphenicol and were capable of carrying out incorporation of ^{14}C-leucine into protein at rates very much greater than had been reported. Taking advantage of these findings, the procedure described in Methods was evolved, yielding preparations of mitochondria and synaptosomes, as shown in Table I. The mitochondria were sensitive to D-threo-chloramphenicol but were not inhibited by cycloheximide, emetine, or chloroquine. The insensitivity to cycloheximide and sensitivity to chloramphenicol was also noted in a recent publication by others (15) on mitochondria obtained from osmotic lysates of synaptosomes, followed by purification on discontinuous sucrose gradient centrifugation. We conclude that, with respect to in vitro protein synthesis, except for much

faster rates of synthesis, (the basis for which is unknown) mouse brain mitochondria are not grossly different from mitochondria from other tissues, and that the previously reported sensitivity of brain mitochondria to cycloheximide can be attributed to contamination of these preparations with synaptosomes. And it is important to note that, conversely, the cycloheximide-sensitive activity of synaptosomes cannot, therefore, be attributed to the mitochondria.

On the other hand, synaptosomes were found to be inhibitable by chloramphenicol, cycloheximide, emetine, and chloroquine (Table I).

TABLE I

EFFECTS OF INHIBITORS OF PROTEIN SYNTHESIS UPON BRAIN MITOCHONDRIA AND SYNAPTOSOMES

Additions to Incubations	Mitochondria			Synaptosomes		
	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3
None	11,740	12,013	19,200	3,350	3,525	3,164
Chloramphenicol (CAM)	1,360	2,177	6,125	1,154	1,207	1,520
Cycloheximide (Cyclo)	11,900	11,080	22,375		1,533	2,483
Emetine (EME)		11,750	20,450	1,633		2,160
Chloroquine	11,209		18,700	1,930	2,420	
CAM & Cyclo						1,262
CAM & EME						1,343
Cyclo & EME						2,160

Incubations were in a shaking water bath at 30° for 40 minutes. The incubation flasks contained, in a total volume of 1 ml: 15mM $MgCl_2$; 5mM $NaPO_4$, pH 7.6; 130mM KCl; 70mM Bicine buffer, pH 7.6; 0.2mM ATP; 5mM K-succinate; and 0.5 µc of ^{14}C-L-Leucine, specific activity 278 µc/µm; either mitochondria or synaptosomes suspended in 0.33 M sucrose; and (when added, as shown) 100 µg D-*threo*-chloramphenicol, 100 µg cycloheximide, 65 µg emetine hydrochloride, or 0.5mM chloroquine. All results are CPM/mg-protein.

Based upon the reported effect of chloroquine in retarding entry of amino acids into *T. pyriformis* (21) and taking into account our observations cited

above, it is possible that the inhibition of synthesis by chloroquine is secondary to a prevention of the entry of ^{14}C-leucine into the sites of synthesis within the synaptosome.

In many experiments the sum of the emetine- or cycloheximide-sensitive activity and the chloramphenicol-sensitive activity was greater than could be accounted for on the basis of two independent protein synthesizing systems. Furthermore, as shown in Table I, the activity inhibitable by cycloheximide in the presence of chloramphenicol was less than in its absence. And, of course, the converse of this statement is true: chloramphenicol inhibited less activity in the presence of cycloheximide than when chloramphenicol was the only inhibitor present.

Although one cannot, with this much information, state that there is a sequential series of steps in the overall protein synthetic activity, the data clearly are not compatible with two completely independent systems as Morgan (8) thought might be the case. Indeed, this suggestion is in line with current speculation, reviewed in depth by Ashwell and Work (17), on the general question of the interdependence that most probably exists between mitochondrial and cytoplasmic protein synthetic systems.

In summary, we believe that the isolated synaptosome is of interest not only because of the real possibility that local synthesis in the synaptic region is taking place, but it may, in addition, afford a relatively simple system for study of interactions between the mitochondrial and nonmitochondrial synthetic systems.

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