# PROTEIN SYNTHESIS IN BRAIN MITOCHONDRIAL AND SYNAPTOSOMAL PREPARATIONS

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The anomolous protein synthesis, sensitive to cycloheximide, which has been observed in brain mitochondrial and synaptosomal preparations, has been studied. It is concluded that this protein synthesis is due to the presence, as a contaminant in both preparations, of a ribosome-containing particle which contains soluble enzymes and is limited by a plasma membrane.

## 1. Introduction

Since the initial observation by Yellin, Butler and Stein [1], several authors [2–5] have noted that protein synthesis in brain mitochondrial preparations (BMPS) is relatively insensitive to chloramphenicol (CAP) and is sensitive to cycloheximide (CH). Initially Gordon and Deanin [2] reported that BMPS was completely resistant to CAP, but experiments carried out by other workers [1, 3–5] have demonstrated CAP-sensitive BMPS under conditions where significant bacterial contamination was excluded. Furthermore, their results could be interpreted to indicate a slight (10%) inhibition of BMPS by CAP. But the unexpected observation of CH-sensitive BMPS is more difficult to explain.

It is well-known that even after purification by sucrose density gradient centrifugation, there is considerable contamination of brain mitochondrial preparations with synaptosomes [6]. Since several groups have demonstrated that protein synthesis in synaptosomal preparations (SPS) is CH-sensitive [2, 7–9], it appeared possible that this contamination could account for the unusual characteristics of BMPS.

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## 2. Methods

Brain mitochondria were prepared by several methods [10–15], and synaptosomes were prepared by the method of Kurokawa, Sakamoto and Kato [14]. Incubations were performed in various media [2, 7, 16, 17]. Essentially similar results were obtained in all cases. The results reported in this paper were obtained with mitochondria prepared by the method of Gray and Whittaker [10] and incubations were performed under the conditions of Gordon and Deanin [2]. CAP, CH and RNAase were added to the incubation media at concentrations of 100 $\mu$g/ml.

Incubations were performed, and the uptake of $^{14}$C-leucine into protein determined as previously described [18]. For the determination of the label in soluble and particulate protein, incubations were terminated by the addition of an equal vol. of ice-cold incubation medium containing 10 mM leucine, and the particles sedimented at 20,000 $g$ for 15 min. The pellet was vigorously suspended in distilled water, then centrifuged at 100,000 $g$ for 1 hr. The soluble and particulate proteins were treated as above.

## 3. Results and discussion

As can be seen from table 1, the responses of BMPS and SPS to CAP and CH are remarkably

Table 1
Inhibition of brain mitochondrial and synaptosomal protein synthesis by cycloheximide and chloramphenicol.

| | Synaptosomes | | | Mitochondria | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Chloramphenicol | 20 | 23 | 25 | 25 | 11 | 0 | 23 | 40 | 56 |
| Cycloheximide | 71 | 77 | 88 | 65 | 90 | 43 | 49 | 30 | 50 |
| RNAase | 0 | 0 | 15 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | [2] | [8] | | [2] | [3] *a* | [3] *b* | [4] | [5] |

Results are presented as percent inhibition of protein synthesis. Figures in brackets refer to the reference from which the results are quoted. *a* and *b* refer to high sodium and high potassium media respectively. Data from [2] were calculated from the means of the ranges presented.

similar. The observation of CAP-sensitivity is in contradiction with the results of Gordon and Deanin [2], but is in accord with the results of numerous other workers [1, 3–5, 8]. It has been reported that the effect of CAP on BMPS is dependent upon the potassium ion concentration of the incubation medium [3], but even using a high potassium incubation medium [16], Gordon and Deanin [2] failed to observe an inhibition. Whatever the explanation of their failure, there is now little doubt that BMPS is CAP-sensitive.

Nevertheless the CH-sensitive protein synthesis remains to be explained. As shown in table 2, this is not the only unusual characteristic of BMPS, Quabain inhibits BMPS, despite the apparent absence of ouabain-inhibited ATPase from brain mitochondria [19]. This inhibition suggests the operation of a protein synthesizing system limited by a plasma membrane (cf. the results obtained with SPS [20]). The ionic conditions necessary for SPS and BMPS are in agreement with this hypothesis, since both are maximal under the extracellular condition of a high sodium ion concentration, whereas mitochondrial protein synthesis requires high potassium.

Similarly it is difficult to explain the inhibition of BMPS by 2-deoxyglucose. Brain mitochondria when highly purified contain only low activities of most glycolytic enzymes [13], and generally these activities are attributed to synaptosomal contamination. However SPS has been shown to be dependent upon production of ATP by the action of glycolytic enzymes, the TCA and oxidative phosphorylation [21]. No requirement for exogenous energy substrates was observed for BMPS and SPS in these experiments or in those of Gordon and Deanin [2], in contrast to mitochondria [22].

Mitochondrial protein synthesis *in vitro* preferentially labels the insoluble proteins [23]. In these experiments, as in those of Haldar [5], there was a high specific activity of label in the soluble protein. The labelling of the soluble protein was CH-sensitive [5], as was the case for SPS [7].

In summary, the properties of BMPS are remarkably similar to those reported for SPS [7]. The CH-sensitive activity observed cannot be attributed to

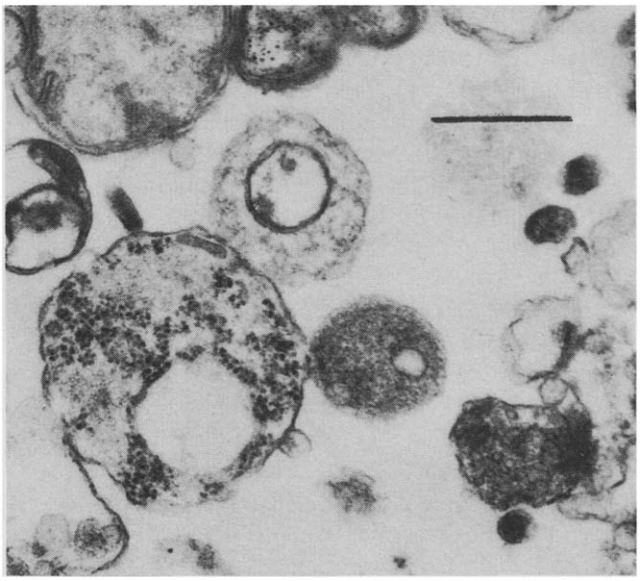

Fig. 1. Electron micrograph of a synaptosomal fraction prepared by the method of Kurokawa, Sakamoto and Kato [14]. The black bar represents 0.5 μm.

Table 2
Characteristics of brain mitochondrial and synaptosomal protein synthesis.

| | BMPS | SPS |
|---|---|---|
| Ouabain (% inhibition) | 52 | 60 |
| 2-Deoxyglucose (% inhibition) | 71 | 71 |
| Substrate requirement | none | none |
| Label in soluble protein (specific activity soluble/ total specific activity) | 0.91 | 0.84 |
| % Label in soluble protein | 26 | 30 |
| Ionic requirements | high sodium | high sodium |

Protein synthesis was measured as described in Methods. Ouabain (0.5 mM) and 2-deoxyglucose (10 mM) were added to the basic incubation medium.

simple ribosomal contamination since RNAase had no effect on the protein synthesis. But the results obtained are consistent with the operation of two systems: (a) a eukaryotic ribosomal system, protected from RNAase by a membrane; (b) a normal mitochondrial system.

The eukaryotic ribosomal system was believed to be within the synaptosome on the basis of numerous properties common to the hypothetical particle and the synaptosome [7]. However, very rare particles containing ribosomes have been reported in synaptosomal preparations (fig. 1, ref. [24]), and similar particles have been observed in brain mitochondrial preparations (Yellin, personal communication). These particles are sufficient to account for the CH-sensitive protein synthesis observed. Their origin is not known, but they could derive from glial cells [24]. Or perhaps from growing axons, where ribosomes have been observed [25], although ribosomes are absent from the normal axon [26].

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