MITOCHONDRIAL CONTRIBUTION TO PROTEIN SYNTHESIS IN CEREBRAL CORTEX

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SUMMARY

Emetine (at 100 μg/ml) blocks the incorporation of ^{3}H-leucine into the total protein of slices from rat cerebral cortex by > 95%; the block on the synthesis of cytosol proteins is quantitative. Under these conditions protein synthesis resistant to emetine is localized in a mitochondrial fraction that is sensitive to chloramphenicol at 100 μg/ml to the extent of ~ 70%. We infer that some 15% of the proteins of cerebral cortex mitochondria formed during a 60 min period have been provided by their own protein synthesizing system.

INTRODUCTION

Examination of the current literature dealing with protein synthesis in rodent brain brings out several puzzling and unresolved features. a) While incorporation of labeled amino acids in vivo or in slices is reported to be highly sensitive to the glutarimide antibiotics such as cycloheximide (CH) or its derivatives (1, 2, 3), the analogous process catalyzed by purified ribosomes (4, 5) or polysomes (6) is relatively refractory to this type of inhibition. b) While there is some disagreement concerning the extent of inhibition of incorporation by CAP in slices [ranging from about 40% according to (6 and 7), to none or a slight stimulation according to (2 and 3)] the confusion becomes compounded when the action of this inhibitor is studied with different preparations of isolated mitochondria: values have been reported that vary from 60% at 83 μg/ml (7) to ~ 0% at 100 μg/ml (5); a tabulation of these and other data has been presented by Morgan (8). c) Conversely, the sensitivity of incorporation by these same particles to CH is variously claimed to be virtually complete [≥ 90% at 5-40 μg/ml (5)] or sensibly absent [< 10%

at concentrations below 250 μg/ml (7)], with other values falling between these two extremes (8).

This is not a satisfactory state of affairs for investigators interested in using these (otherwise highly selective) inhibitors of cytoribosomal and mitoribosomal protein synthesis (reviews in 9, 10) in studies designed to assess the contributions of these two synthetic systems to the biogenesis of mitochondrial and other membranes in brain tissue. A possible means of resolving the dilemma is suggested by the recent report of Perlman and Penman (11) that emetine (EME), an inhibitor first introduced by Grollman (12), although similar to the glutarimide antibiotics in mode of action, has proved to be a far more effective selective inhibitor of cytoribosomal protein synthesis in HeLa cells.

We have been able to show that in slices from rat cerebral cortex EME (at 100 μg/ml) blocks the incorporation of ^{3}H-leucine into total protein by > 95% while the block on the synthesis of cytosol proteins is quantitative. Under these conditions EME-resistant protein synthesis is localized in a mitochondrial fraction that is sensitive to CAP (at 100 μg/ml) to the extent of ~ 70%. The data suggest that some 15% of the protein of cerebral mitochondria synthesized during the incubation period originates in an EME-resistant, CAP-sensitive system, presumably the mitoribosomes localized within the particle. We can also infer that under the same conditions the bulk of cerebral proteins are synthesized by the EME-sensitive system also responsible for the formation of the soluble proteins, presumably constituted by the various populations of cytoribosomes, and that mitoribosomes do not contribute in any significant fashion.

MATERIALS AND METHODS

We used 60 day male Sprague-Dawley rats obtained from Harlan Industries, Inc. Animals were killed by decapitation, the cortex isolated and tissue slices prepared by the method of McIlwain (13) as modified by White (3). In general, slices obtained from one animal were used per experimental point.

After a pre-incubation of 25 min, in the presence of appropriate drugs wherever indicated, in 10 ml of a Krebs Ringer, low Ca^{2+} medium (13, 14), ^{3}H-leucine (sp. act. = 55.2 Ci/mmole, supplied by New England Nuclear) was added to the concentrations indicated, and incubation continued for 60 min. The gas phase was pure O_2 throughout; incorporation was terminated by the addition of 3 ml of ice-cold medium and immediate sedimentation, followed by resuspension in 10 ml of medium and sedimentation (90 s for the two steps). Each preparation was then washed with 10 ml of cold 0.32 M sucrose (pH 7.4), homogenized in another 10 ml of 0.32 M sucrose and fractionated into crude nuclear ($\underset{\sim}{P_1}$), mitochondrial ($\underset{\sim}{P_2}$), microsomal ($\underset{\sim}{P_3}$) and soluble ($\underset{\sim}{S}$) fractions by the method of Whittaker (15) as modified by Cotman and Mahler (16). The crude mitochondrial fraction was subjected to osmotic shock by repeated passage through a 14 gauge needle and homogenization in 5 ml of distilled H_2O (pH 7.4) and centrifuged through 30 ml of 1.4 M sucrose for 90 min in a SW-27 rotor at 25,000 rpm in a Beckman Model L2-65B centrifuge. In this manner (17) we obtained a crude membrane fraction at the top of the gradient ($\underset{\sim}{T}$) as well as a mixed (cell-body plus synaptosomal) mitochondrial one in the pellet. Its further characterization and properties after separation of the mitochondrial components will form the subject of a separate communication.

Aliquots of the various tissue fractions were removed for determination of their protein content (18) and radioactivity (19). Two separate disks were prepared for each fraction and counted by liquid scintillation techniques for periods of time sufficient to reduce the counting error to < 5%. These replicate samples agreed to within ± 5% of their mean.

RESULTS AND DISCUSSION

The results of three separate experiments are shown in Table 1; the agree ment is satisfactory. In another experiment, not shown, analogous results were obtained when the incubation period was shortened to 30 min. In earlier would result in an estimate that is too high. In the absence of information concerning the quantitative aspects we have chosen not to take these factors

into account. We also believe that the small amount of EME-insensitive incorporation found in all particulate fractions is not due to anything more

TABLE 1

INCORPORATION OF ^{3}H-LEUCINE INTO FRACTION OF RAT CORTEX SLICES

Expt. No.	Conditions	Specific Activities (cpm/mg) H	P_1	P_3	S_3	T	M
1 a	Control	3241 3142	3347 2485	4566 4732	8204 6254	2313 2393	1564 1946
b	EME	169 135	193 126	101 94	BG	57 54	276 342
c	EME + CAP	120 111	88 103	130 100	BG	60 67	114 92
d	(b/a) x 100%	4.7	5.5	2.2	0.4	2.2	18.0
e	(c/a) x 100%	3.6	3.3	2.5	0.6	2.8	6.0
2 a	Control	5621	6529	6353	9746	4211	2753
b	EME	252 246	239 222	182 166	BG	173 144	496 483
c	EME + CAP	144 137	174 189	170 144	BG	90 87	203 194
d	(b/a) x 100%	4.7	3.5	2.7	0.2	3.8	17.7
e	(c/a) x 100%	2.5	2.8	2.4	< 0.1	2.2	7.3
3 a	Control	7774	7461	9322	9842	4886	4154
b	EME	398 437	373 366	413 389	BG	222 238	907 915
c	EME + CAP	222 232	216 246	239 236	BG	110 118	223 224
d	(b/a) x 100%	5.3	5.0	4.3	0.3	4.7	22.0
e	(c/a) x 100%	3.0	3.0	2.6	0.4	2.4	5.3

All incubations as described in the text on Dubnoff shaker at 100 strokes/min for 60 min uder O_2; ^{3}H-L-leucine used: 50 μCi in Expt. 1, 100 μCi in Expt. 2. Medium contained (13, 14) in μmoles/ml: NaCl (127), KCl (5.1), K_2HPO_4 (1.3), $CaCl_2$ (0.75), $MgSO_4$ (1.3), glucose (10), phosphate buffer, pH 7.4 (10). All inhibitors at 100 μg/ml, except for CAP in expt. 1 (50 μg/ml). In expt. 3 an 100-fold excess of unlabeled leucine was added at 60 min, and incubation continued for 10 min longer. Every set (horizontal line) constitutes a separate incubation.

mysterious than unavoidable contamination by mitochondria and mitochondrial fragments. Calculation shows that, given a mitochondrial contribution of some 20-25% to the total homogenate protein (15, 17, 23, 24), the value of the EME-resistant component due to this contribution alone should be of the order of 4-5%; this is the value found.

The actual extent of the mitoribosomal contribution to the synthesis of organelle protein shown here is within the range determined by the analogous technique with CH on unicellular eucaryotes (20, 25). Like these results, the current ones suffer from the quantitative uncertainties introduced by uncoupling what is normally a tightly coupled, interdependent system (10, 20). Unlike them it suffers from the additional uncertainty that, since the incubation time is short relative to the half-life of the particle, the contribution found may not be due to the class of mitochondrial proteins in general but to a subclass constituted by those proteins synthesized or renewed more rapidly than the rest.

Finally the results together with those published recently by Bosmann and Hemsworth (7) and by Morgan (8) make unnecessary additional postulates or hypotheses concerning special and unusual properties of brain mitochondria with regard to either cerebral protein synthesis or their own biogenesis (5, 25, 26).

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All incubations as described in the text on Dubnoff shaker at 100 strokes/min for 60 min under O_2; ^{3}H-L-leucine used: 50 μCi in Expt. 1, 100 μCi in Expts. 2 and 3. Medium contained (13, 14) in μmoles/ml: NaCl (127), KCl (5.1), $CaCl_2$ (0.75), $MgSO_4$ (1.3), glucose (10), phosphate buffer, pH 7.4 (10). All inhibitors at 100 μg/ml, except for CAP in expt. 1 (50 μg/ml). In expt. 3 an 1000fold excess of unlabeled leucine was added at 60 min, and incubation continued for 10 min longer. Every set (horizontal line) constitutes a separate incubation. Drugs did not alter the amount of acid soluble label taken up by the slices.

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