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PROTEIN SYNTHESIS ON FREE AND ENDOPLASMIC RETICULUM-BOUND POLYSOMES OF RAT BRAIN*

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The question of metabolic differences between the ribosomes attached to the endoplasmic reticulum of mammalian cells and those not so attached (bound and free ribosomes) has been examined in liver[1-6], kidney[7,8] and spleen[9,10] as well as in hepatomas[11] and the tissue partition ratios[12] and the RNA turnover[8,13] of the two populations have been investigated. It was also shown recently that bound ribosomes carry out the synthesis of hepatic glycoprotein[14] to the virtual exclusion of free ribosomes[15] and that the nutritional state[16] and the age of the animal[17] are likely to determine the relative proportions of bound vs. free ribosomes as well as the momentary contribution of each population to cellular protein synthesis. Conversely, it has been shown[10] that, in rat spleen, the bound and the free ribosomes are identical immunologically and in several other respects.

Since suitable procedures which do not use detergents have recently become available for the isolation of bound[18-20] and free polysomes[21] from brain tissue, it seemed of interest to compare the in vivo protein synthetic capacity of both populations. This communication reports results of experiments on the synthesis of pulse-labelled protein by bound and free polysomes of rat cerebral cortex.

Materials and Methods

Immature rats (12-13 days old), whose brain cortex contains bound and free

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polysomes[22], were injected intrathecally in groups of 3, each with 50 µl (6.5 µC) of [^{14}C]-L-leucine, [^{14}-C]-L-arginine or [^{14}-C]-L-phenylalanine (UL, s.a.> 250 mc/mmole, ICN Co., Irvine, California). Two, five and ten min later, the animals were killed and the specific radioactivity (counts/min per mg of protein) of the polysome-associated labelled, nascent protein[20] was determined in several, fractions which were isolated by different centrifugal procedures (Table I).

Results

The results (expts. No. 1, 4 and 6, Table I) demonstrate that the cesium procedure[18] yields bound polysomes of 4-5 times the specific activity of the homogenate. The relationship of synthesis to the duration of the *in vivo* pulse is shown in fig. 1. The top curve in the left panel shows that when [^{14}C]-leucine was the precursor, the pulse-dependent increase of the specific activity of bound polysomes was greater than that of free polysomes[21] (middle curve). It should be noted that the bound polysomes isolated by the procedure of ref. 21 exhibited specific activities which were uniformly lower than those of the free polysomes (expts 2, 5 and 7, Table I). It became possible, however, to observe precursor-dependent variations in specific activity of bound polysomes after removal of all of the endoplasmic reticulum membranes (see right panel, fig. 1). This led to the isolation of polysomes of higher specific activity than that of the corresponding free polysomes, when [^{14}C]-leucine was the precursor (expt. No. 2, Table I), but which had a lower specific activity than the corresponding free polysomes after the administration of arginine or phenylalanine (expts. 5 and 7, Table I). The results of experiment 3, Table I show that the specific activity of the bound polysomes isolated by the procedure of Ref. 21 may be increased by a factor of 2 by selectively removing the agranular component of the endoplasmic reticulum with the cesium procedure[18]. An additional illustration of the effectiveness of this step as a means of enhancing the specific activity of the bound polysomes, is provided by the middle panel of fig 1 which compares the labelling patterns of the total (middle curve) and, separately, of the granular (top curve) and the agranular components

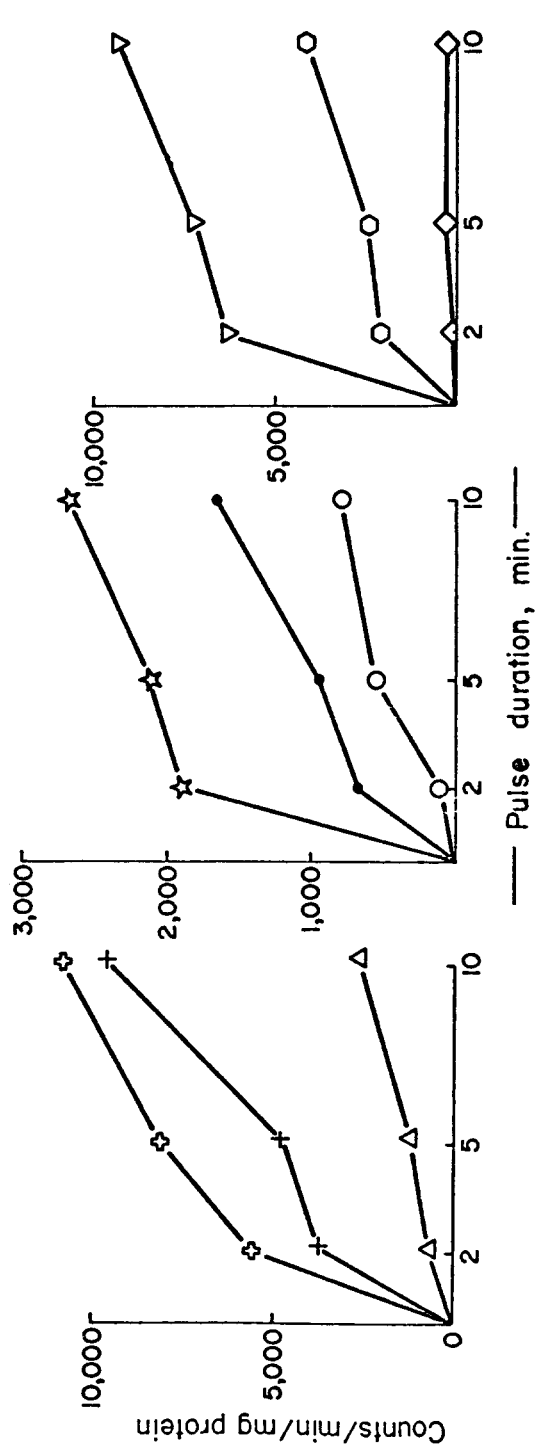

FIG. 1

Left panel: brain cortex homogenates (Δ-Δ) from rats injected with 6.5 μc of [^{14}C]-leucine were processed, at the times indicated, as outlined in ref. 21 to give free polysomes (++) and as in ref. 18 to give endoplasmic reticulum-bound ribosomes (✠). The radioactivity [insoluble in 5% trichloroacetic acid, retained on Millipore filters (0.45 μ)] and protein content were determined as described elsewhere[20]. Middle panel: the procedure was identical to that described in the experiment No. 3 of Table I. The suspension of bound polysomes (●-●) (also containing the agranular endoplasmic reticulum) was pelleted and was processed as in ref. 18 to separate the granular (✠) from the agranular (0-0) endoplasmic reticulum. Right panel: the procedure was as described for experiment No. 2 of Table I. The gradient band containing the total endoplasmic reticulum (◇) (Ref. 21) was treated with sodium deoxycholate (0.2%) and the clarified solution was centrifuged at 269,000 x g for 35 min to give a pellet (▽) and a supernatant (◇).

TABLE I

The specific activity of free and endoplasmic reticulum-bound polysomes.*

Expt No.	[^{14}C]-precursor	Procedure (ref. No.)	Fraction Analyzed	specific activity (counts/min per mg protein)
1	leucine	18	homogenate	1,940
			bound polysomes	9,550
2	leucine	21	free polysomes	4,840
			bound polysomes	2,480
			bound polysomes after deoxycholate	7,120
3	leucine	21	bound polysomes	964
		followed by		
		18	bound polysomes	2,104
4	arginine	18	homogenate	658
			bound polysomes	3,073
5	arginine	21	free polysomes	8,600
			bound polysomes	2,780
			bound polysomes after deoxycholate	7,750
6	phenylalanine	18	homogenate	1,990
			bound polysomes	8,260
7	phenylalanine	21	free polysomes	14,050
			bound polysomes	3,360
			bound polysomes after deoxycholate	7,740

* The duration of the *in vivo* pulse was 5 min

The endoplasmic reticulum-bound and free polysomes were isolated as follows: Experiment No. 1: the postmitochondrial supernatant (refs. 18, 19) was sedimented at 104,000 x g for 60 min yielding the microsomal pellet which was suspended by manual homogenization in a solution containing 20 mM Tris, pH 7.2, 10 mM Mg acetate (solution E of ref. 18) and 15 mM CsCl and the suspension (1.7 ml) was layered on 3.3 ml of 1.3 M sucrose containing 15 mM CsCl. The tubes were centrifuged in the SW-50L rotor at 204,000 x g for 3 hr. The pellet contains the bound polysomes. Experiment No. 2: the free polysomes were obtained by the procedure of ref. 21. The endoplasmic reticulum-bound polysomes

were collected from the 0.5-2.0 M sucrose interface (see ref. 21) and their radioactivity and protein content were determined[18-20] on an aliquot. The remainder of the suspension was treated with sodium deoxycholate (0.2%, w/v, 10 min at 0°) and the clarified solution was centrifuged at 269,000 x g for 35 min. The pellet consists of ribo-and polysomes freed of membranes. Experiment No. 3: the procedure of ref. 21 was followed up to the point where the bound polysomes (plus the agranular endoplasmic reticulum) were collected from the 0.5-2.0 M sucrose interface. The material was pelleted (269,000 x g, 35 min), the pellet suspended in solution E (see expt. No. 1) containing 15 mM CsCl and the suspension was processed as in expt. No. 1. Experiment No. 4: as expt. No. 1; Experiment No. 5: as expt. No. 2; Experiment No. 6: as expt. No. 1; Experiment No. 7: as expt. No. 2.

(bottom curve) of the endoplasmic reticulum. It may be seen that the agranular component, which contains approximately 13% of the total endoplasmic reticulum RNA[19] and 43% of the protein[19], incorporated relatively little radioactivity under the conditions of the experiments.

The present results indicate that both free and endoplasmic reticulum-bound polysomes actively participate in protein synthesis, as it occurs in the cerebral cortex of the immature rat. Preliminary results (O.Z. Sellinger and W.G. Ohlsson, unpublished observations) confirm this finding for subcortical tissue and the cerebellum as well. The results do not support the conclusions of Nievel and Cumings[23] who suggested that "ribosomes bound in the membrane (are) the principal site of protein synthesis in the brain". Experiments are in progress[24] in which cerebral protein synthesis is being examined in terms of differences in the quality of the products manufactured on free and bound polysomes.

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