PREFERENTIAL EFFECT OF OESTRADIOL 17β IN VIVO ON THE PROTEIN SYNTHETIC ACTIVITY OF UTERINE MEMBRANE-BOUND RIBOSOMES

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SUMMARY: Membrane-bound ribosomes were isolated and quantified in uterine tissue of ovariectomized rats. Oestrogen stimulation of {^{14}C}phenylalanine incorporation on free and membrane-bound ribosomes was 70 % and 134 % respectively. The addition of poly U to the assay mixture reduced the hormonal stimulation of incorporation to about 23 % in both classes of ribosomes. Membrane-bound ribosomes were relatively less responsive to increase in {^{14}C}phenylalanine incorporation due to the addition of poly U. Hormone treatment resulted in an increased proportion of membrane-bound ribosomes. The results suggest that membrane--bound ribosomes are more responsive to hormonal stimulus than are free ribosomes in rat uterus.

INTRODUCTION

A considerable body of evidence suggests that oestrogens, such as oestradiol 17β , administered to immature or ovariectomized adult rats increase the translational activity of uterine ribosomes (1-4). The stimulatory effect of oestradiol 17β on uterine protein synthesis reaches a maximum at 10-12 h after hormone treatment (1,2), and is accompanied by the appearance of newly synthetized cytoplasmic ribosomes (5-7). Ribosomes exist in most animal tissues in two topographic situations: free and membrane-bound (8,9). Available data have led credence to the view that free and membrane-bound ribosomes selectively translate different templates and that microsomal membranes may play a role in regulating the translation of the attached ribosomes (9,10).

Previously reported effects of oestradiol on protein synthesis in uterus have been concerned with isolated total ribosomes and unfractionated microsomal fraction (1-3). The present investigation was undertaken to isolate free and membrane-bound ribosomes from rat uterus and to examine whether the known effect of

oestradiol in stimulating protein synthesis involved free or membrane-bound ribosomes or both. The results show that the hormone preferentially stimulates endogenous protein synthetic activity in membrane-bound ribosomes. The oestrogen treatment also enhances the relative proportion of membrane-bound ribosomes in uteri of the ovariectomized rats.

MATERIALS AND METHODS

Female rats of the Wistar strain (180-200 g) were used. They were ovariectomized 3-4 weeks prior to use. Oestradiol 17β (10 µg, Sigma Chemical Co., USA) was given as a single tail vein injection in 0.5 ml of isotonic saline containing 5 % ethanol. The control rats received the solvent only. The source of the rest of the material has been described elsewhere (11,12).

For the preparation of free and membrane-bound ribosomes from rat uterus, the animals were killed by decapitation and unruptured uteri were removed immediately. These were trimmed to remove fat and connective tissue and were weighed. The uteri were then minced and suspended (1 ml/uterus) in a medium containing 50 mM Tris-HCl (pH 7.5), 25 mM KCl, 5 mM $MgCl_2$, 1 mM dithiothreitol and 0.3 mg/ml of poly(vinyl sulphate). Homogenization of the tissue was accomplished by 2.5 min treatment on Ultra-Turrax tissue disintegrator. The homogenate was filtered through two layers of muslin cloth, and centrifuged at 10 000 x g for 10 min at 4 °C. The nuclear-mitochondrial pellet was saved for further fractionation. The post-mitochondrial supernatant fraction was layered over a discontinuous sucrose gradient that consisted of 4 ml of 1.3 M sucrose overlaid on 3 ml of 2 M sucrose having the same ionic composition as that of the homogenization medium. The gradients were centrifuged at 149 000 x g for 24 hours. Free ribosomes formed a pellet at the bottom of the tube. The interphase layer was removed, diluted and centrifuged at 149 000 x g for 60 min to obtain membrane-bound ribosomes. The first nuclear-mitochondrial pellet was further subfractionated to recover membrane-bound ribosomes that may have sedimented with nuclei and mitochondria (13). The combined bound ribosomal fractions were purified to remove any lysosomal contamination (14). The ribosomal fractions were diluted so that equal amounts of ribosomes were used for cell-free amino acid incorporation assay.

RNA, protein and protein radioactivity were determined as described previously (11,12).

The assay for amino acid incorporation was carried out in a final volume of 0.2 ml of a mixture containing: 50 mM Tris-HCl (pH 7.5), 80 mM NH_4Cl, 10 mM $MgCl_2$, 0.05 mM 19 unlabelled amino acids, 1 mM dithiothreitol. 10 mM creatine phosphate, 5 µg of phosphokinase, 1 mM ATP, 0.4 mM GTP, 0.5 mg pH 5 enzyme protein, 0.4 mg partially purified transferases, 1 µC of {^{14}C}phenylalanine (sp. act. 513 mCi/mmole) and 10 µg ribosomes (given as RNA). Poly U (100 µg) was present in the incubation mixture where indicated. The concentrations of cations, pH 5 enzyme and transferases were experimentally found to be optimal for promoting amino acid incorporation into polypeptide. Incubation was carried out at 37 °C for 30 min. The preparation of pH 5 enzyme and transferases have been described (11).

RESULTS

The method used for the isolation of membrane-bound ribosomes gives near-quantitative results since it employes relatively low centrifugal force to sediment the first nuclear--mitochondrial pellet that was further fractionated to recover membrane-bound ribosomes sedimenting with this fraction (13). The RNA/protein ratio of free and membrane-bound ribosomes averaged 0.73 and 0.12 respectively.

Prior to testing membrane-bound ribosomes for amino acid incorporative activity, they were purified free of lysosomal contamination (14). Such contamination may result in lower amino acid incorporation by membrane-bound ribosomes. Table I compares uterine free and membrane-bound ribosomes from control and 12 h oestradiol-treated animals for {^{14}C}phenylalanine incorporation in the presence and absence of 100 µg poly U. Hormone treatment results in a 60 % increase in endogenous amino acid incorporative activity of free ribosomes. Oestrogen effect on the endogenous activity of membrane-bound ribosomes was very much amplified; the hormonal stimulation of this fraction was 134 % compared with the control (Table I). When the amino acid incorporation was carried out in the presence of saturating

TABLE I. EFFECT OF OESTRADIOL TREATMENT IN VIVO ON THE PROTEIN SYNTHETIC ACTIVITIES OF FREE AND BOUND MEMBRANE-RIBOSOMES

Source of uteri	{^{14}C}phenylalanine incorporated counts/min x 10^{-3} per mg ribosomal RNA			
	Free ribosomes		Bound ribosomes	
	- Poly U	+ Poly U	- Poly U	+ Poly U
Control rats	20.5	54.2	23.0	55.5
Oestradiol-treated rats	32.3	66.5	54.1	68.1

Ovariectomized rats were injected with oestradiol (10 µg) or with vehicle only, and killed 12 h later. Free and membrane--bound ribosomes were isolated from uteri as described in the text. Following their incubation in a cell-free amino acid incorporation system at 37 °C for 30 min (see Methods), hot trichloroacetic acid-insoluble radioactivity was determined. The figures represent the mean of three experiments.

amount of exogenous messenger poly U, both free and membrane--bound ribosomes showed equal stimulation (about 23 %) as a result of hormone treatment.

An important feature that emerges from the results shown in Table I is the poly U effect viz. additional {^{14}C}phenylalanine incorporated over and above endogenous incorporation in free and membrane-bound ribosomes after oestrogen treatment. Free ribosomes from control and hormone-treated animals show 170 % and 106 % stimulation, respectively, by poly U. On the other hand, the poly U effect in membrane-bound ribosomes decreases from 141 % in control to 26 % in treated rats. Thus the hormone treatment decreases the ability of both free and membrane-bound ribosomes to respond to poly U. The reduced response to poly U after hormone treatment was strikingly more marked in membrane-bound ribosomes.

In order to study whether or not free and membrane-bound ribosomes respond differently in initiating new polypeptide chains, two well-known inhibitors of the initiation step (16,17),

TABLE II. EFFECT OF INHIBITORS OF INITIATION OF PROTEIN SYNTHESIS ON THE ACTIVITIES OF UTERINE FREE AND MEMBRANE-BOUND RIBOSOMES

Fraction	% inhibition of {^{14}C}phenylalanine incorporation	
	NaF (10 mM)	Aurintricarboxylic acid (0.03 mM)
Free ribosomes from control animals	31 (30-32)	20 (19-22)
Free ribosomes from oestradiol--treated animals	35 (31-39)	25 (22-26)
Bound ribosomes (control)	32 (28-35)	19 (17-21)
Bound ribosomes (oestradiol)	48 (46-51)	33 (30-35)

The conditions of incubation etc. were the same as those described in legend to Table I except that the reaction mixture also contained the above-indicated inhibitors. Poly U was not included in the assay mixture. The figures represent the mean of three experiments (with range in parentheses).

NaF and aurintricarboxylic acid were employed. The results shown in Table II reveal that there is a significant increase in inititation of new polypeptide chains on membrane-bound ribosomes derived from oestrogenized animals. This increase is less marked in free ribosomal fraction.

When actinomycin D (10 μg/20 g body weight) was administered 10 min before the hormone-treatment, the observed stimulation in amino acid incorporation in vitro after 12 h was abolished by 50-60 % in both free and membrane-bound ribosomes (data not shown).

Table III shows the results of an experiment designed to see possible changes in the proportion of uterine membrane-bound ribosomes after treatment of ovariectomized rats with oestradiol 17β. In control uteri, 14-17 % of the recovered material was membrane-bound. After treatment with oestrogen for 12 h the proportion of membrane-bound ribosomes increased to values ranging from 20-25 %. This increase was reproducible in all individual experiments. At 4 h after hormone treatment there was no change in proportion of membrane-bound ribosomes compared with control (data not shown). Increasing the time for oestradiol treatment from 12 to 24 h did not result in a further increase in the proportion of membrane-bound ribosomes (Table III).

TABLE III. CHANGES IN PROPORTION OF MEMBRANE-BOUND RIBOSOMES AFTER TREATMENT WITH OESTRADIOL IN VIVO

	Membrane-bound ribosomes (% of total ribosomal population)			
	Control		Oestradiol treated	
Experiment No.	12 h	24 h	12 h	24 h
1	15.5	16.8	22.7	21.5
2	17.3	17.0	25.4	26.0
3	14.0	14.4	20.5	23.6

Oestradiol 17β (10 μg) was administered to ovariectomized rats 12 and 24 h prior to use. Control rats received the vehicle only. Free and membrane-bound ribosomes were isolated from the trimmed uteri as described under Materials and Methods.

DISCUSSION

Previously published reports (1-4) on the stimulatory effects of oestradiol 17β on protein synthesis in uterine tissue have been mainly concerned with unfractionated microsomal fraction or with total ribosomal population (free + membrane-bound ribosomes). Thus Teng and Hamilton employed rather high centrifugal force (27 000 x g for 15 min) to sediment the nuclear-mitochindrial pellet and recovered ribosomes from the postmitochondrial supernatant fraction thus obtained. Considerable amount of membrane-bound ribosomes are lost under these conditions of isolation (15). To the author's knowledge, there is no study where free and membrane-bound ribosomes have been isolated and quantified in the uterine tissue and examined for their protein synthetic activities in vitro after oestrogen treatment of the rat in vivo.

The results presented demonstrate that, under the influence of oestrogen, membrane-bound ribosomes are stimulated to a much greater extent than are free ribosomes, in endogenous mRNA--directed incorporation of amino acids into protein. This observation is in keeping with the results obtained by Korner (18) with livers of hypophysectomized rats after treatment with growth hormone. Working with a non-mammalian system, Tata has shown that administration of tri-iodothyronine to immature Rana catesbeiana tadpole results in a co-ordinated increase in RNA and membrane phospholipid synthesis and that there is an enhanced rate of amino acid incorporation in vivo on the rough membranes (19). It is interesting to note that, in the present study, the stimulation of protein synthesis after oestradiol treatment is accompanied by an increased proportion of membrane-bound ribosomes in uterus. There is an increase of 80-90 % in the absolute amount of ribosome at 12 h after oestrogen treatment (results not shown; cf. 1).

Oestrogen treatment results in a lower response to stimulation by exogenous synthetic messenger of amino acid incorporation on both classes of ribosomes, but the decrease in poly U effect is more apparent on membrane-bound ribosomes. The poly U effect may indicate the relative endogenous template activity of a given ribosomal preparation (20). Thus it appears that preferential stimulation of membrane-bound ribosomes could be,

at least in part, due to an increase in endogenous messenger RNA associated with this fraction. A lower response to poly U in target tissues after treatment with oestrogen and testosterone has been observed previously (2,21).

Taken together, the results presented suggest that membrane-bound ribosome are relatively more responsive to the hormonal stimulus. Recent evidence suggests that messenger RNA after migrating to cytoplasm becomes associated with the cytoplasmic membranes and that the attachment of ribosomes and newly transported messenger RNA may be concurrent events (23,24). Whether preferential increase in membrane-bound ribosome activity is due to their greater stability (12,22) or selective and more efficient translation of newly synthesized messenger RNA after hormone treatment, remains to be elucidated.

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