Regulation of Polyribosome Formation and Protein Synthesis in the Uterus

EFFECT OF ADMINISTRATION OF GRADED DOSES OF OESTRADIOL-17 β AND ACTINOMYCIN D ON THE AMINO ACID-INCORPORATION ACTIVITY *IN VITRO* AND THE CYTOPLASMIC CONCENTRATION *IN VIVO* OF POLYRIBOSOMES

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1. The effects of graded doses of oestradiol-17 β and actinomycin D, administered separately or together, on the amino acid-incorporation activity *in vitro* and the cytoplasmic concentration *in vivo* of uterine polyribosomes are described. Preparations of polyribosomes isolated from uteri of ovariectomized adult rats were determined for cytoplasmic concentration *in vivo* and assayed for [¹⁴C]leucine-incorporation activity in the cell-free system, exactly as described by Teng & Hamilton (1967b). 2. A minimal dose of 10 μ g of oestradiol-17 β administered for 10 h was found to increase, by about 100%, both the amino acid-incorporation activity *in vitro* and the cytoplasmic concentration *in vivo* of the polyribosomes. A minimal dose of 250 μ g of actinomycin D administered for 10 h was found to inhibit, by about 50%, the incorporation activity *in vitro* of the polyribosomes. All doses of the inhibitor administered for 10 h failed to alter the cytoplasmic concentration *in vivo* of the polyribosomes. 3. A dose of 10 μ g of oestradiol-17 β restored to the control value the inhibitory effect of a dose of either 50 or 125 μ g of actinomycin D on the activity *in vitro* of the polyribosomes, at 10 h after treatment with the inhibitor and the hormone. In these experiments, there was an increase of 60-100% in the cytoplasmic concentration *in vivo* of the polyribosomes. 4. A dose of 125 μ g of actinomycin D, administered to animals along with 10 μ g of oestradiol-17 β for 6-36 h, abolished the hormone-induced enhancement of the incorporation activity *in vitro*, but did not prevent an increase of about 200% in the cytoplasmic concentration *in vivo* of the polyribosomes. However, treatment with 750 μ g of the inhibitor abolished both stimulatory effects of the hormone. 5. The results reported indicate that the stimulatory effects of oestradiol-17 β *in vivo* on the number and activity of the cytoplasmic polyribosomes in the uterus of the ovariectomized rat have different sensitivities to actinomycin D, but the primary molecular mechanisms responsible for the results are unknown. The major conclusion drawn is that the formation and appearance in the cytoplasm of newly formed polyribosomes are important features of the early action of oestrogen in the uterus.

Polyribosome formation and protein synthesis have been shown to be regulated by oestrogen acting in the uterus of the ovariectomized adult rat (Teng & Hamilton, 1967b). Both the rate of amino acid incorporation *in vitro* and the cytoplasmic concentration *in vivo* of the polyribosomes were increased 100% at 12 h after treatment with a single dose of oestradiol-17 β . These increases were abolished if the animals used were treated with actinomycin D 1 h before hormone treatment. We concluded that the effect of oestrogen on genetic trans-

lation in the organ was a consequence of preceding effects on genetic transcription. This conclusion is in agreement with investigations that demonstrate sequential stimulations of synthesis of RNA and of protein by many growth-promoting and developmental hormones acting in their characteristic tissues (for reviews see Segal & Scher, 1967; Tata, 1967; Hamilton, 1968).

We now describe the effects of administration of graded doses of oestradiol-17 β and actinomycin D, separately and together, on the amino acid-incorporation activity *in vitro* and the cytoplasmic concentration *in vivo* of uterine polyribosomes. We

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demonstrate that the two stimulatory effects of the hormone have different sensitivities to actinomycin D. By treatment with an appropriate dose of the inhibitor, it is possible to block only that effect of the hormone which enhances the amino acid-incorporation activity of the polyribosomes. In this instance, the stimulatory effect of the hormone on the formation of ribonucleoprotein particles and their transport to and accumulation in the cytoplasm as polyribosomes remains unperturbed.

MATERIALS AND METHODS

Most of the materials and methods employed have been described previously (Teng & Hamilton, 1967a,b). Polyribosomal preparations from uteri of ovariectomized female Sprague-Dawley rats, each weighing 180–200 g, were isolated by procedure 3 and assayed for [14 C]leucine-incorporation activity in the cell-free system incubated for 30 min at 37°C, exactly as described by Teng & Hamilton (1967b). The cytoplasmic concentration of the polyribosomal preparation was assayed by determining the amount, in μ g, of the preparation isolated per mg of DNA in the whole-tissue homogenate, also as described. The recovery of total microsomal ribonucleoprotein in all polyribosomal preparations isolated was about 60%. The remaining details of experimentation and procedure were as described in the relevant figures.

RESULTS

Effect of treatment with graded doses of hormone.

Fig. 1 shows the effect of graded doses of oestradiol-

17 β on the [14 C]leucine-incorporation activity *in vitro* and the cytoplasmic concentration *in vivo* of the polyribosomes isolated from the uterus of the ovariectomized rat, at 10 h after treatment. Both the incorporation activity and the cytoplasmic concentration of the preparation were increased to a maximum of about 100% after treatment with a dose of 10 μ g of the hormone. Treatment with smaller or larger doses of the hormone caused smaller increases in the activity and cytoplasmic concentration of the polyribosomes. The dose-response curve for the stimulation by oestradiol-17 β of the incorporation activity of the polyribosomes thus paralleled closely that observed for the increase of their concentration in the cytoplasm. Fig. 1 also shows that the dose-response curve for the effect of oestradiol-17 β on the weight of the uterus paralleled the curves observed for the biochemical parameters. Treatment with 10 μ g of the hormone caused a maximum increase of about 120% in uterine weight, and treatment with larger or smaller doses of the hormone caused smaller increases. Tata (1966) has previously discussed the evidence for a variety of growth-promoting hormones that a response to a large dose of hormone can be the opposite of that produced by a smaller, presumably more physiological dose.

Effect of treatment with graded doses of inhibitor.

Fig. 2 shows that graded doses of actinomycin D administered to the ovariectomized rat for 10 h did not alter the cytoplasmic concentration of the

Fig. 1. Effect of graded doses of oestradiol-17 β *in vivo* on the [14 C]leucine-incorporation activity *in vitro* (●) and the cytoplasmic concentration *in vivo* (▲) of the polyribosomes isolated from the uterus of the ovariectomized rat. All experimental animals received, intraperitoneally, the dose of hormone indicated dissolved in 0.1 ml of propane-1,2-diol. Control animals received 0.1 ml of the solvent without hormone. Each group consisted of six animals. All animals were killed 10 h after treatment. The uteri were excised, blotted on a damp cloth and weighed immediately. The range of variation in the uterine wet weights determined for each group was 10% or less of the average value. The polyribosomes of each group of pooled uteri were isolated by procedure 3, determined for their cytoplasmic concentration *in vivo*, and assayed for incorporation activity in the cell-free system, as described in the Materials and Methods section and by Teng & Hamilton (1967b). ■, Average weight of uteri.

Fig. 2. Effect of graded doses of actinomycin D *in vivo* on the [^{14}C]leucine-incorporation activity *in vitro* (●) and the cytoplasmic concentration *in vivo* (▲) of the polyribosomes isolated from the uterus of the ovariectomized rat. All experimental animals received, intraperitoneally, the dose of inhibitor indicated dissolved in 0.1 ml of propane-1,2-diol. All animals were killed 10 h after treatment. The remaining details of experimental procedure were as described in Fig. 1. ■, Average weight of uteri.

uterine polyribosomes *in vivo*, whereas their [^{14}C]leucine-incorporation activity *in vitro* was decreased. The incorporation activity of the polyribosomes was inhibited about 40% after treatment with a dose of 125 μg of the inhibitor, and a dose of 250 μg resulted in a maximal inhibition of about 50%. Increasing the dose of inhibitor administered to 1.0 mg caused no further decrease in the incorporation activity. The weight of the uterus, like the organ's cytoplasmic concentration of polyribosomes, was unaltered by any dose of actinomycin D administered. The failure of actinomycin D to cause a decrease in the cytoplasmic concentration of the polyribosomes in the uterus of the ovariectomized adult rat may be explained by the relatively slow rate of generation of new ribosomes as well as the stability of the relatively few ribosomes present in the cytoplasm of the atrophied organ (Moore & Hamilton, 1964; Teng & Hamilton, 1967b). In tissues metabolically more active and possessing a higher cytoplasmic concentration of ribosomes, as in rat liver (Revel, Hiatt & Revel, 1964) or the uterus of the normal rat in the oestrous state (T. H. Hamilton, unpublished work), the inhibitor administered *in vivo* results in a decrease in the cytoplasmic concentration of the ribosomes.

Combined effect of treatment with graded doses of hormone and inhibitor. Initially we examined the effects of administration of graded doses of oestradiol-17 β , together with a dose of either 50 or 125 μg of actinomycin D, at 10 h after treatment. Administration to the ovariectomized rat of 50 μg of the inhibitor alone caused a decrease of 30% in the

[^{14}C]leucine-incorporation activity of the polyribosomes *in vitro*, and no alteration of their cytoplasmic concentration *in vivo* (Fig. 3a). Administration of 1, 10, or 100 μg of the hormone 20 min after treatment with 50 μg of the inhibitor resulted in a restoration of the incorporation activity of the polyribosomes. A dose of 10 μg of the hormone was most effective in countering the inhibitory effect of 50 μg of actinomycin D, and restored the incorporation activity to 110% of the control value. A dose of 100 μg of the hormone was less effective in restoration of the decreased incorporation activity due to the inhibitor alone. This observation is in accordance with the decreased effect on the incorporation activity of the larger dose of the hormone administered alone (see Fig. 1).

The cytoplasmic concentration of uterine polyribosomes was increased about 100% by treatment with either 1 or 10 μg of hormone and 50 μg of the inhibitor (Fig. 3b). Again, a dose of 100 μg of the hormone was less effective than the smaller doses in increasing the cytoplasmic concentration of the polyribosomes (cf. Figs. 1 and 3a).

At 10 h after treatment with 125 μg of actinomycin D alone, the incorporation activity of the polyribosomes was decreased by 40% (Fig. 3b). A dose of 10 μg of oestradiol-17 β administered 20 min after treatment with the inhibitor caused an almost complete restoration of the incorporation activity. However, larger doses of the hormone were progressively less effective in this restoration. A dose of 100 μg of the hormone administered shortly after treatment with the inhibitor caused a decrease in

Fig. 3. Combined effect of graded doses of oestradiol-17 β *in vivo* and of 50 μ g (a) or 125 μ g (b) of actinomycin D *in vivo* on the [14 C]leucine-incorporation activity *in vitro* (●) and the cytoplasmic concentration *in vivo* (▲) of uterine polyribosomes. All animals received the inhibitor 20 min before the dose of hormone indicated. All animals were killed 10 h after the initial treatment. The other details of experimental procedure were as already described., Incorporation activity of the polyribosomes after treatment with propane-1,2-diol alone; ----, incorporation activity after treatment with the inhibitor alone.

Fig. 4. Effect of treatment *in vivo* with 125 μ g of actinomycin D, with or without treatment *in vivo* with 10 μ g of oestradiol-17 β , on the [14 C]leucine-incorporation activity *in vitro* (a) or the cytoplasmic concentration *in vivo* (b) of uterine polyribosomes. Animals receiving hormone were treated 20 min after administration of the inhibitor at zero-time. All animals were killed at the time indicated. ●, ▲, Polyribosomes from animals receiving actinomycin D and oestradiol-17 β ; ○, △, polyribosomes from animals receiving the inhibitor alone; ■, average weight of the uteri of animals receiving both inhibitor and hormone.

the incorporation activity to a value below that resulting from treatment with the inhibitor alone. In these experiments, the cytoplasmic concentration of the polyribosomes was increased about 60% by all treatments with hormone in the presence of the inhibitor.

Fig. 4 shows the effects of administration of 125 μ g of actinomycin D and 10 μ g of oestradiol-17 β , either separately or together as a function of time after treatment. At 12 and 24 h after treatment with actinomycin D alone, the incorporation activity of the uterine polyribosomes was decreased by about 40% (Fig. 4a), in accordance with the result of preceding experiments (see Fig. 2). At 36 h after treatment with the inhibitor, there was a small but repeatable rise in the incorporation activity. The cytoplasmic concentration of the polyribosomes was again unaltered by treatment with the inhibitor alone (cf. Figs. 2 and 4b). Administration of the hormone 20 min after treatment with the inhibitor caused no stimulation of the incorporation activity, although the activity was returned to that of the control value at 6–12 h after treatment. From 12 to 36 h, however, there was a decrease of about 35% in the incorporation activity.

The cytoplasmic concentration of the polyribosomes was increased markedly under these conditions of treatment with the inhibitor and the hormone. At 36 h, when the stimulatory effect of the hormone on the incorporation activity of the polyribosomes was completely abolished (Fig. 4a), their concentration in the cytoplasm was increased nearly 200% (Fig. 4b). The weight of the uterus increased in this experiment, but to a lesser extent than if treated with the hormone alone (cf. Figs. 1 and 4b).

Previous work in this laboratory had demonstrated that larger doses of actinomycin D, from 250 to 750 μ g administered per animal, abolished the stimulatory effect of the hormone on the cytoplasmic concentration of the polyribosomes (Teng & Hamilton, 1967b) or on the rate of synthesis of

Fig. 5. Effect of treatment *in vivo* with 750 μ g of actinomycin D, with or without treatment *in vivo* with 10 μ g of oestradiol-17 β , on the [14 C]leucine-incorporation activity *in vitro* (a) or the cytoplasmic concentration *in vivo* (b) of uterine polyribosomes. The experimental details were identical with those described in Fig. 4, except for the larger dose of inhibitor administered. ●, ▲, Polyribosomes from animals receiving actinomycin D and oestradiol-17 β ; ○, △, polyribosomes from animals receiving the inhibitor alone; ■, average weight of uteri of animals receiving both inhibitor and hormone.

monomeric ribosomes (Moore & Hamilton, 1964) in the uterus. The experiments described in Fig. 4 were repeated with the animals receiving 750 μ g of actinomycin D for several intervals of time to 18 h. This dose of the inhibitor abolished not only the stimulatory effect of the hormone on the incorporation activity of the polyribosomes (Fig. 5a), but also the increase in their cytoplasmic concentration and the increase in the weight of the uterus (Fig. 5b).

DISCUSSION

In our experiments, doses of 125 μ g or less of actinomycin D were mostly not lethal, whereas animals treated with larger doses of 500 μ g to 1.0 mg of the inhibitor usually died between 24 and 36 h. These animals exhibited a comatose condition shortly after treatment, and at death showed haemorrhagic digestive tracts and excessive abdominal fluid. The suggestion has been made that actinomycin D causes increased release of corticosteroid hormones by the adrenal gland *in vivo*, and that these steroids, rather than actinomycin D, are responsible for the inhibition of oestrogen-stimulated RNA synthesis in the uterus (Lippe & Szego, 1965). However, Nicolette & Mueller (1966) demonstrated that in ovariectomized and adrenalectomized animals actinomycin D abolished most of the

oestrogen-stimulated RNA synthesis in the uterus.

Actinomycin D, not unexpectedly, causes a variety of metabolic perturbations *in vivo* (Tata, 1966). The inhibitor causes in particular a marked disaggregation of hepatic polyribosomes into monomeric ribosomes in the rat or mouse (Blobel & Potter, 1967; Sarma, Reid & Sidransky, 1969). In the investigations cited relatively large and probably lethal doses of about 50 μ g or more of the inhibitor per 20 g of body weight were administered. The majority of our experiments employed smaller and sublethal doses of about 12.5 μ g or less of the inhibitor per 20 g of body weight. Also, our experiments examined the effect of the inhibitor on the oestrogen-induced formation of polyribosomes in the previously atrophied and ribosomes-deficient uterus of the ovariectomized rat (Moore & Hamilton, 1964; Teng & Hamilton, 1967b).

The nucleolus is generally considered the major site of synthesis of ribosomal RNA and of ribonucleoprotein particles programmed for transport to the cytoplasm for formation of polyribosomes (Busch, Byvoet & Smetana, 1963; Perry, 1963; McConkey & Hopkins, 1964). Actinomycin D is known to be a strong and preferential inhibitor of nucleolar synthesis of ribosomal RNA (Reich & Goldberg, 1964; Trakatellis, Axelrod & Montjar, 1964). It is now evident that a major early effect of growth-promoting hormones, e.g. oestrogen, androgen, or thyroid hormone, is the stimulation of synthesis of ribosomal RNA and the formation of ribosomes (Tata, 1966; Hamilton, 1968; Liao, 1968). The inhibitory effects of actinomycin D on oestrogen-induced formation of polyribosomes *in vivo* (Figs. 3 and 5) probably result from a blocking of nucleolar RNA synthesis and of the transport to the cytoplasm of ribonucleoprotein particles.

We have at present no adequate explanation for our observation that a relatively small and sublethal dose of actinomycin D administered *in vivo* will abolish the effect of oestrogen on the amino acid-incorporation activity *in vitro* of uterine polyribosomes, but not the stimulatory effect on their formation and appearance in the cytoplasm. The mechanism of stimulation by various hormones of the amino acid-incorporation activity of ribosomes has been interpreted in several ways, but in no instance has a precise molecular mechanism been described (for review see Manchester, 1968). These stimulations have been attributed to increased rates of synthesis of mRNA and thus increased concentrations of mRNA in the ribonucleoprotein particles isolated (Liao & Williams-Ashman, 1962), to an unspecified 'coding' of ribosomes (Tata & Widnell, 1966; cf. Byrne, Levin, Bladen & Nirenberg, 1964), and to alterations in the structure of ribosomes or a 'loading' of their protein or other components (Rampersad & Wool, 1965; Earl &

Korner, 1966; Teng & Hamilton, 1967b; Hamilton & Teng, 1969). Conceivably our finding that the stimulatory effect of oestrogen on polyribosome formation in the uterus can be dissociated from the effect on polyribosome activity by treatment with a sublethal dose of actinomycin D might prove useful in future studies of the mechanism whereby the hormone controls genetic translation in the cytoplasm. This statement seems particularly warranted in light of the recent investigations demonstrating effects of hormone treatment or withdrawal on specific subunits of ribosomes obtained from mammalian skeletal muscle or liver, apparently independent of the concentration of mRNA (Martin & Wool, 1968; Barden & Korner, 1969).

Finally, our present observations are consistent with our previous conclusion (Teng & Hamilton, 1967b) that the appearance in the cytoplasm of new polyribosomes having different template or structural properties or both for amino acid incorporation is an important aspect of the early action of oestrogen in the uterus. We note moreover that the stimulation by oestrogen of the amino acid-incorporation activity of uterine polyribosomes always occurs concomitantly with an increased appearance of new polyribosomes in the cytoplasm, and that this appearance of new polyribosomes in the cytoplasm is always correlated in turn with an increase in the weight of the organ (Fig. 1). Thus our observations are also compatible with the current concept that regulation of protein synthesis by various growth-promoting and developmental hormones is largely geared to the appearance in the cytoplasm of newly formed ribosomes (Tata, 1968).

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