A Very Rapid Effect of Androgen on Initiation of Protein Synthesis in Prostate

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Communicated by Elwood V. Jensen, December 5, 1974

ABSTRACT The initiation of protein synthesis by ribosomal particles of rat ventral prostate was studied by measuring ribosomal binding of an initiator [35S]methionyl-tRNA. The binding activity is dependent on ribosomes, GTP, and a prostate cytosol protein fraction. The 40S but not the 60S ribosomal subunit particles are active. The cytosol activity decreases rapidly within one hour after the rat is castrated. This loss is prevented by an intraperitoneal injection of 17β-hydroxy-5α-androstan-3-one (5α-dihydrotestosterone). The cytosol activity can be stimulated almost immediately (<10 min) after an intravenous injection of low dose (15 μg per rat) of 5α-dihydrotestosterone into the castrated rat.

Testicular androgens are required to maintain the normal protein-synthesizing activity of ribosomes in rat ventral prostate (1). The androgen effect appears to be preceded by an increase in nuclear RNA synthesis (2). There are indications that androgens, like other steroid hormones (3, 4), act by binding to specific receptor proteins and that the complexes formed migrate into the prostate cell nuclei, where they appear to regulate gene transcription (2, 5).

It has also been suggested in recent years that certain steroid receptors may also have a more direct effect on the post-transcriptional processes involved in gene expression (2, 6–9). Although this possibility has not been well explored experimentally, we have shown that androgen- and estrogen-receptor complexes can associate with the nuclear ribonucleoprotein and ribosomal subunit particles in the target cells (8, 9). We report here also that orchietomy of rats can result in a rapid decrease in the ability of the cytosolic factors to support the initiation of protein synthesis by ribosomal particles of ventral prostate, and that this decrease is very rapidly reversed by an injection of dihydrotestosterone into the androgen-deficient animals.

MATERIALS AND METHODS

[35S]Methionine (30–100 Ci/mmol) was purchased from New England Nuclear. 17β-Hydroxy-5α-androstan-3-one (5α-dihydrotestosterone) was obtained from Steraloid. tRNA was prepared from rat liver by phenol extraction and was deacylated as described by Von Ehrenstein and Lipmann (10). Rat liver tRNA was charged with [35S]methionine by Escherichia coli synthetase, which acylated only the formylatable species that are required for the initiation of protein synthesis (11, 12).

Manipulation of Animals. Sprague–Dawley, male rats (body weight, 300 g) were castrated via the scrotal route during ether anesthesia. When 5α-dihydrotestosterone was administered in vivo, the control animals were given the same amount of solvent carrier. Sesame oil and 0.9% (w/v) NaCl containing 10% (v/v) ethanol were used as the solvent carriers for the intraperitoneal and intravenous injections, respectively. Animals were killed by cervical dislocation, the ventral prostate was dissected free of its capsule, and its volume was measured by displacement in an ice-cold medium. For comparison, three to four rats were used in each group. Experiments were repeated at least four times to ensure reproducibility.

Preparation of Prostate Cytosol and Ribosomal Particles. All manipulations were carried out at 0–2°C. The ventral prostate was minced and homogenized in 4 volumes of a medium containing 0.25 M sucrose, 25 mM KCl, 5 mM MgCl₂, 3 mM dithiothreitol, and 20 mM Tris·HCl buffer, pH 7.5 (medium A). The homogenate was centrifuged at 170,000 × g for 1 hr. The supernatant was used as the cytosol fraction.

Prostate ribosomes were obtained from rats castrated 17 hr earlier. The homogenization and centrifugation were carried out as described above. The surface of the sediment was rinsed with medium A. The particulated materials were then suspended in medium A containing 0.33% (w/v) deoxycholate and made up to three times the original tissue volume. The suspension was centrifuged at 10,000 × g for 10 min to remove cell nuclei and mitochondria. A 2.5-ml portion of the supernatant was layered in a centrifuge tube on 1.5 ml of 1.7 M sucrose containing 25 mM KCl, 5 mM MgCl₂, 3 mM dithiothreitol, and 20 mM Tris·HCl buffer, pH 7.5. Ribosomes were pelleted by centrifuging the tube at 300,000 × g for 3 hr in a SW-56 rotor. The surface of the ribosomal pellets was rinsed and the pellets were suspended in medium A. Large aggregated materials, if present, were eliminated by centrifugation at 3000 × g for 5 min. Ribosomal subunit particles were prepared by the method of Blobel and Sabatini (13).

Assay of Ribosomal Binding of [35S]Methionyl-tRNA. The standard assay was carried out in a final volume of 0.3 ml in a reaction mixture containing 90 mM KCl, 5 mM MgCl₂, 3 mM dithiothreitol, 1.3 mM GTP, 20 mM Tris·HCl buffer, pH 7.5, 180 μg (2.16 absorbance units at 260 nm) of prostate ribosomes, and the requisite amounts of cytosol fraction and [35S]methionyl-tRNA. The mixture was incubated at 30°C for 20 min.
10 min, unless indicated otherwise. The reaction was terminated by diluting with 5 ml of an ice-cold medium containing 90 mM KCl, 5 mM MgCl₂, and 20 mM Tris-HCl buffer, pH 7.5 (medium B). The diluted mixture was filtered through a Millipore membrane (type, HA: pore size, 0.45 µm) and the residue retained on the membrane was washed with 5 ml of medium B three times. The radioactivity retained on the filter was measured in a scintillation counter using a scintillation fluid containing toluene-Triton X-100 (3:1), 0.4% (w/v) diphenyloxazole, and 0.005% (w/v) 1,4-bis[2-(5-phenyloxazolyl)]benzene.

RESULTS

Requirements for Ribosomal Binding of [³⁵S]Methionyl-tRNAᵣ. Ribosomal binding of a radioactive initiator aminoacyl-tRNA has been widely used in studies of the initiation of protein synthesis in eukaryotic cells (14-16). In the prostate system (Table 1), the retention of the initiator [³⁵S]methionyl-tRNAᵣ by the Millipore filter was dependent on the presence in the reaction mixture of GTP, a ribosomal subunit, and a cytosol fraction. Other experiments also indicated that the concentrations of Mg²⁺ and KCl used in the standard assay mixture were optimal. The cytosol activity was apparently due to proteins, since the activity could be abolished by heating at 70°C for 10 min but was not lost by precipitation with ammonium sulfate or after an extensive dialysis.

When the ribosomal subunit particles were used in the assay, the 40S but not the 60S fraction was found to be active. This suggests that the formation of the [40S-methionyl-tRNAᵣ] initiation complex can indeed occur in the ventral prostate, as has been concluded for other mammalian systems (17-19).

Effect of Androgen Deprivation. Initially, we examined the effect of castration and androgen administration on the ability of the prostate ribosome to bind [³⁵S]methionyl-tRNAᵣ. Since this method revealed no clearly reproducible differences, we studied the effect of androgen manipulation on the cytosol activity.

In the experiment shown in Fig. 1, the comparison was made by using a prostate ribosomal preparation from castrated animals and the prostate cytosol preparations from both normal rats and rats castrated 17 hr previously. In the absence of a cytosol fraction only a low binding activity was observed, but the addition of a cytosol preparation from normal rats enhanced the initial rate of binding by about 10-fold. Castration resulted in a marked decrease in the cytosol activity.

The effect of castration could be observed when the cytosol fraction was rate-limiting and the binding activity was proportional to the amounts of cytosol present. As shown in Fig. 2, the cytosol activity is lost rapidly, within 1 hr after castration.

Effect of Androgen Replenishment. The intraperitoneal injection of a relatively large dose (mg quantity) of 5a-dihydro-

![Fig. 1. Effect of castration on the activity of the prostate cytosol in controlling the rate of ribosomal binding of [³⁵S]methionyl-tRNAᵣ. The experimental conditions were as in Table 1 except that the binding activity was assayed in the absence (O) and presence of a prostate cytosol fraction from normal rats (●) or rats castrated 17 hr earlier (□). The incubation was carried out for the length of time shown on the abscissa.](image1)

![Fig. 2. Effect of castration on the prostate cytosol activity in facilitating [³⁵S]methionyl-tRNAᵣ binding by prostate ribosomes. The experimental conditions were as in Table 1 except that the incubation was carried out for 10 min. The cytosol fraction was obtained from normal rats or from rats castrated for the length of time shown on the abscissa. The cytosol activities are compared by taking that of the normal rats as 100%. The results of five other experiments were very similar to that shown in this figure.](image2)
testosterone immediately after castration not only prevented the loss, but also enhanced the cytosol activity. The stimulation could be observed within 1 hr after the intraperitoneal injection of the androgen (Fig. 3).

To study further the rapid effect of the androgen, we injected the castrated rats intravenously with a low dose (10–20 μg) of dihydrotestosterone. As shown in Fig. 4, the stimulatory effect was clearly observed within 10 min after the injection. In each of the four experiments so far performed, the effect was greater at 10 min than at 30 min after the injection, indicating that a continuous supply of androgens might be necessary for the maintenance of the cytosol activity.

As shown in Table 2, the effects of castration and androgen injection could also be seen when the assay was performed with a purified preparation of the 40S subunits of the prostate ribosomes in the presence of poly(A,U,G). In this experiment, the androgen effect was still distinctly observable even after more than 90% of the RNA in each of the cytosol preparations had been removed by filtration through a DEAE-cellulose column. These findings suggest that the androgen-sensitive factor(s) involved is a protein(s) that may regulate the formation of the [40S–methionyl-tRNAf] initiation complex.

**DISCUSSION**

Our present study suggests that in ventral prostate, the activity of certain cytosol protein factors that are required for or can regulate initiation of protein synthesis on ribosomes is under strict control of the androgen levels in the experimental animals. In mammalian protein-synthesizing systems, such as reticulocytes (17, 20, 21), liver (22), muscle (23), ascites (24), and fibroblasts (18), the initiation process is known to be mediated by at least three initiation factors. Androgens may

**Fig. 3.** Effect of an intraperitoneal injection of 5α-dihydrotestosterone on the cytosol initiation factors. The prostate cytosol fractions were obtained from normal rats (●), rats castrated 19 hr earlier (○), and castrated rats injected with 5α-dihydrotestosterone (2.5 mg) immediately after castration (△) or 1 hr before the rats were killed (○). The ribosomal binding of [35S]methionyl-tRNAf was carried out as in Table 1 except that incubation was for 10 min with the amounts of the cytosol fractions shown on the abscissa. The activity (21.3 fmol) found in the absence of an additional cytosol fraction was deduced from the data.

**Fig. 4.** Rapid effect of 5α-dihydrotestosterone on the cytosol factors required for ribosomal binding of [35S]methionyl-tRNAf. All rats were castrated 17 hr earlier. 5α-Dihydrotestosterone (15 μg per rat) was injected intravenously into the castrated rats 10 min (●) or 30 min (△) before they were killed. The control castrates (○) received the same amount of the injection medium without the androgen. The incubation was carried out for 20 min with the amounts of the cytosol fractions shown on the abscissa. Other conditions were as in Table 1. The activity (17.6 fmol) found in the absence of an additional cytosol fraction was deduced from the data.

**Table 2.** Effects of castration and injection of 5α-dihydrotestosterone on the cytosol factors that are required for the formation of the [40S–methionyl-tRNAf] initiation complex

<table>
<thead>
<tr>
<th>Rats used for preparation of cytosol</th>
<th>Untreated cytosol</th>
<th>DEAE-cellulose-treated cytosol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>73.0</td>
<td>64.9</td>
</tr>
<tr>
<td>Castrated</td>
<td>43.9</td>
<td>38.5</td>
</tr>
<tr>
<td>Castrated + 5α-dihydrotestosterone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 hr</td>
<td>53.7</td>
<td>62.5</td>
</tr>
<tr>
<td>Castrated + 5α-dihydrotestosterone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 hr</td>
<td>53.9</td>
<td>57.0</td>
</tr>
</tbody>
</table>

The complete assay system contained 0.4 absorbancy unit (at 260 nm) of the 40S ribosomal subunits, 200 μg of cytosol protein, 2 mM GTP, 480,000 cpm of [35S]methionyl-tRNAf (7 μg of tRNA), 10 μg of poly(A,U,G), 5 mM MgCl2, 3 mM dithiothreitol, 25 mM KCl, 20 mM Tris-HCl buffer, pH 7.3. The reaction mixture was incubated at 30° for 40 min. The cytosol fractions were prepared from normal rats, rats castrated 18 hr earlier, or castrated rats injected with 2.5 mg of 5α-dihydrotestosterone intraperitoneally 1 or 2 hr before they were killed. Each of the cytosol preparations was divided into two portions. The KCl concentration of one portion was adjusted to 0.3 M. The adjusted cytosol was allowed to pass through an equal volume of DEAE-cellulose column to remove RNA. More than 90% of the cytosol RNA can be removed by this procedure. After incubation, the reaction mixtures were washed as described in **Materials and Methods** except that the washing medium contained 25 mM KCl.

Our present study suggests that in ventral prostate, the activity of certain cytosol protein factors that are required for or can regulate initiation of protein synthesis on ribosomes is under strict control of the androgen levels in the experimental animals. In mammalian protein-synthesizing systems, such as reticulocytes (17, 20, 21), liver (22), muscle (23), ascites (24), and fibroblasts (18), the initiation process is known to be mediated by at least three initiation factors. Androgens may
affect these known protein factors or others that are yet to be identified. The androgen effect is apparently not due to RNA in the cytosol preparation.

Our observations indicate that the effect of androgen on the activity of the protein initiation factors occurs almost immediately after an androgen enters the target cell. It is not possible to state definitely, at this time, that this rapid effect is not dependent on the new nuclear RNA synthesis. Nevertheless, in several preliminary experiments, we found that high doses of actinomycin D (500 μg per rat) in vivo were not able to reduce the androgen-stimulated cytosol activity. From an in vitro study, it may be possible to delineate clearly whether the steroid hormone or its cellular receptor can regulate the protein synthesis directly, as has been considered (4, 8).

Note Added in Proof. We have found that the factor affected is a cytosol protein that binds methionyl-tRNA. The androgen effect can be abolished by anti-androgens that antagonize receptor binding of androgens. The androgen-receptor complex, therefore, appears to have a rather immediate effect on the initiation of protein synthesis.

We thank Miss Pamela A. Chudzinski for her skillful technical assistance. This study was supported by Grant BC-151 from the American Cancer Society, Inc. and Grants AM-09461 and HD-07110 from the U. S. National Institutes of Health. The work was presented in the Fourth International Congress on Hormonal Steroids, Mexico City, September, 1974.