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RAPID HOMOLOGOUS UP-REGULATION OF BINDING CAPACITY OF ANDROGEN RECEPTORS IN INTACT CELLS

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Incubation of MCF 7 cells with 5 α-dihydrotestosterone (DHT) at 37°C led to a 70% increase in the Bmax of androgen receptor, as compared to the values measured at 2°C, without detectable changes in equilibrium dissociation constants. When MCF 7 cells were incubated with hormone at 2°C, to reach steady-state levels of androgen-receptor complex, a subsequent temperature shift to 37°C induced a rapid ($t_{\frac{1}{2}}$=3 min) cycloheximide-insensitive increase in DHT binding to androgen receptor. MCF 7 cell treatments at 37°C either before or after incubation with DHT at 2°C showed that up-regulation of binding capacity of androgen receptor could be observed only if hormone is present during incubation at physiological temperature.

Steroid hormone receptors represent trans-acting transcription factors whose normal function is strictly dependent on their association with ligand (1). Hormone binding capacity of receptor proteins could then represent a potential target of control mechanisms involved in tissue responsiveness and modulation of steroid hormone action (2). This concept was proposed after evidence accumulated on the existence of a very rapid ($t_{\frac{1}{2}}$=3-5 min) turnover of steroid binding capacity of glucocorticoid (3-5), and androgen (6) receptors in intact cells. Still, a direct proof of the existence of signals which control the binding capacity of steroid receptors in intact cells has not been obtained. We here report that the binding capacity of androgen receptors in intact MCF 7 cells is rapidly increased upon hormone treatment at physiological temperatures through activation of preexisting receptor proteins.

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EXPERIMENTAL PROCEDURES

Materials. 5 α-dihydro [1,2,4,5,6,7-^{3}H(N)] testosterone (118.5 Ci/mmol) was purchased from New England Nuclear. All other reagents were of analytical grade.

Cell culture conditions. MCF 7 cells were grown in 5% carbon dioxide in air at 37°C in Petri dishes with a culture medium composed of Dulbecco's Modified Eagle Medium containing antibiotics (100 units/ml penicillin, 100 μg/ml streptomycin, and 25 μg/ml gentamicin), 1% non essential amino acids, 6 ng/ml bovine insulin and 10% foetal calf serum. Four days before the experiment, cells were seeded in the culture medium lacking phenol red and containing 10% charcoal-stripped (7) foetal calf serum. On the day of the experiment, cells were harvested by treatment for 5 min at room temperature with 0.25% trypsin, 5 mM EDTA in 20 mM phosphate buffer, pH 7.4 containing 0.15 M NaCl (PBS).

Measurement of binding capacity of androgen receptors in MCF 7 cells. Cells were washed twice by suspension in PBS and centrifugation for 8 min at 600xg, and were dispersed in Dulbecco's Modified Eagle Medium lacking phenol red and containing 50 mM HEPES buffer, pH 7.3. Cell suspensions were then incubated as specified in the text with the indicated concentrations of tritiated DHT and in the presence or absence of a 200-fold molar excess of nonradioactive DHT as competitor. At the end of the incubations, cells were brought to 2°C and washed three times by suspension in PBS and centrifugation for 8 min at 600xg. Cells were then lysed by suspension in 1 ml of 20 mM Tris-HCl, pH 7.5 at 2°C, 1.5 mM $MgCl_2$, 10 mM NaCl, containing 3 mg/ml of digitonin, and incubation for 30 min at 2°C with occasional vortexing. The homogenates were then brought to a final 0.6 M NaCl concentration, and cellular androgen-receptor complexes were extracted by incubation for 45 min at 2°C (6) and centrifugation for 30 min at 16,000xg. The supernatants of this centrifugation were then treated for 10 min at 2°C with a dextran-coated charcoal pellet (8) to remove free steroid, and aliquots were taken for measurements of bound radioactivity (6). Specific DHT binding to androgen receptors was determined by subtraction of binding obtained in the presence of competitor from that found in its absence.

RESULTS AND DISCUSSION

In preliminary experiments we performed analyses of DHT binding to androgen receptors in intact MCF 7 cells at 2 and 37°C (Fig. 1). Scatchard transformation of specific binding data revealed the presence of androgen receptor whose K_D was 1.5 $\pm$ 0.5 and 1.2 $\pm$ 0.3 x 10^{-9} M (n=3) when cells were incubated at 2 and 37°C, respectively. In spite of almost identical equilibrium dissociation constants, the B_{max} measured at 37°C (489 $\pm$ 189 fmol/mg DNA, n=3) was 70% higher than that found at 2°C (293 $\pm$ 100 fmol/mg DNA, n=3).

We then considered the possibility that the increased levels of androgen-receptor complex measured at 37°C, as compared to 2°C, might have been due the fact that steady-state levels of binding were not attained at

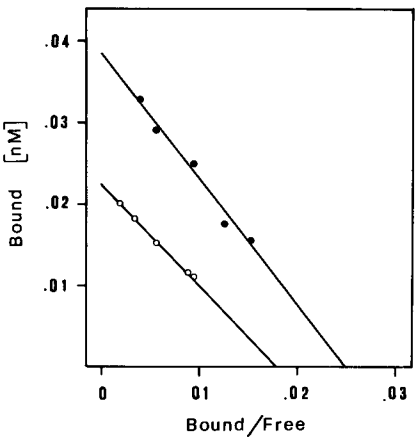

Figure 1. Analysis of DHT binding in intact MCF 7 cells at 2 and 37°C. MCF 7 cell suspensions received increasing amounts of tritiated DHT either in the presence or absence of a 200-fold molar excess of unlabeled DHT as competitor, and samples were incubated for 1.5 hr at 2°C followed by 30 min at either 2 (O) or 37°C (●). Specific DHT binding to androgen receptor was determined, and data were subjected to Scatchard transformation (9).

low temperature in the 2 hr incubation we have performed. This did not appear to be the case, however, as maximal binding was reached within 30 min of cell incubation with tritiated DHT at 2°C (Fig. 2), and we could conclude that MCF 7 cell incubation with hormone at 37 °C leads to an increase in the B_{max} of androgen receptor without changes in its K_D.

In order to ascertain whether the increase in DHT binding upon cell incubation at 37°C might depend on ongoing synthesis of androgen receptor, we treated cell suspensions with 5 nM tritiated DHT either in the presence or in the absence of a cycloheximide concentration which inhibits protein synthesis by at least 95% (6), and measured the time-course of steroid binding to androgen receptor at 37°C. The results we obtained (Fig. 3) showed that the temperature shift induced a rapid ($t_{\frac{1}{2}}$=3 min) increase in DHT binding to androgen receptors, which was not suppressed by cycloheximide. We could then conclude that incubation of MCF 7 cells at 37°C resulted in the activation of steroid binding capacity of preexisting androgen receptor molecules.

We had then to consider the possibility that in the course of cell harvesting, a temporary impairement of normal energy supply might have induced a partial inactivation of androgen receptors, whose binding capacity was recovered upon cell incubation at 37°C (6). In order to

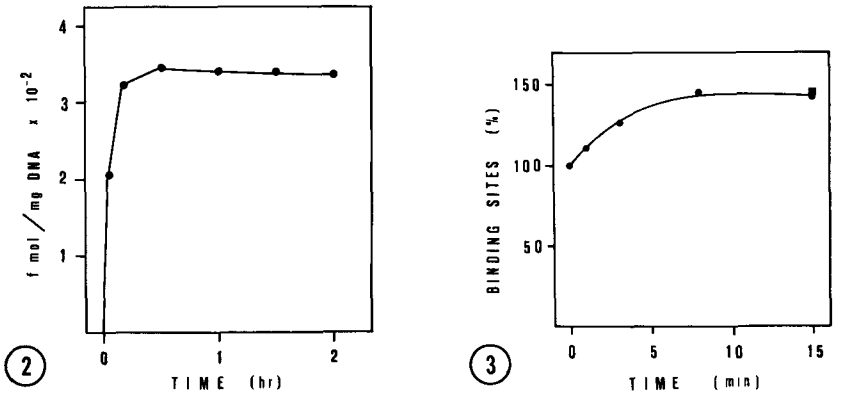

Figure 2. Time-course of DHT binding to androgen receptors in MCF 7 cells at 2°C. Cell suspensions were incubated at 2°C with 5 nM tritiated DHT either in the presence or absence of a 200-fold molar excess of nonradioactive DHT. At the indicated times aliquots were taken and processed to determine specific DHT binding to androgen receptors.

Figure 3. Time-course of DHT binding to androgen receptors in MCF 7 cells upon a temperature shift from 2 to 37°C. Cell suspensions were preincubated for 1.5 hr at 2°C with 5 nM tritiated DHT either in the presence or absence of a 200-fold molar excess of nonradioactive DHT, and with (■) or without (●) 1 mM cycloheximide. At the end of the preincubation, samples were incubated at 37°C, and at the indicated times aliquots were taken and processed to determine specific DHT binding to androgen receptors, which has been expressed as the percentage of that found in cell suspensions maintained for 2 hr at 2°C.

directly test this possibility, the binding capacity of androgen receptors was evaluated in MCF 7 cells which were treated at 37°C either before or after addition of tritiated DHT. The results we obtained, showed that the binding capacity of androgen receptors measured when DHT was present during cell treatment at 37°C was 144.2 ± 5.1% (n=3) of that found in cells maintained at 2°C (control cells). If paired cell suspensions were incubated at 37°C in the absence of hormone, instead, the binding capacity of androgen receptor was 110.4 ± 4.0% (n=3) of the levels found in control cells.

These observations are consistent with the existence of post--translational mechanisms which up-regulate androgen receptors available to hormone binding in target tissues.

A hormone-dependent activation of steroid binding capacity of estrogen receptor has been obtained under cell-free conditions (10), and it has been shown to involve phosphorylation of receptor proteins (10,11). Thus, in the light of our original observations (6), and a recent report on the rapid

hormone-dependent phosphorylation of androgen receptors in intact cells (12), it could be speculated that up-regulation of binding capacity of androgen receptors in MCF 7 cells involves phosphorylation of receptor proteins. Although available evidence on phosphorylation of steroid receptors in intact cells (10-15) would favour the analogy among different receptor systems, the concept of modulation of binding capacity of steorid hormone receptors mediated by phosphorylation deserves further scrutiny, as binding capacity of progesterone receptors does not appear to depend on receptor phosphorylation (16). Thus, it cannot be excluded that the activation of binding capacity of steroid receptors might actually depend on intracellular concentrations of low molecular weight components (17,18).

While we have no information with regard to the molecular mechanism(s) involved in the phenomenon we have described, it should be pointed out that it requires both DHT supply and a physiological temperature. The first characteristic implies that the process is controlled by the hormone itself, which may act through its receptor or some other as yet unidentified component(s). For instance, as the increase in binding capacity of androgen receptor depends on a temperature-sensitive step, we cannot exclude that some enzyme(s) might be involved in this process through modifications of receptor proteins themselves, or the production of a modulator of binding capacity of androgen receptors. The post--translational, homologous up-regulation of active androgen receptors, in any case, shows that 5α-dihydrotestosterone can modulate the binding capacity of its own receptors by non-transcriptional events in target cells.

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