Androgen Effects Mediated by Estrogen Receptor in 7,12-Dimethylbenz(a)anthracene-induced Rat Mammary Tumors

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ABSTRACT

To improve our understanding of the effects of androgens on the induction and growth of mammary tumors, the interaction of androgens on the estrogen receptor (ER) has been evaluated in the 7,12-dimethylbenz(a)anthracene-induced mammary tumor. In vitro competitive experiments have shown that androgens interact on ER with a low affinity but have a good stereospecificity for C19 steroids bearing a 17β- and/or 3β-hydroxyl group. The administration of one dose (≥20 mg) of 5α-dihydrotestosterone to 1-day ovariectomized rats induced ER nuclear translocation in 92% of the mammary tumors. In 60% of these tumors, this translocation was followed by a significant stimulation of the rate of [3H]leucine incorporation into cytosol proteins by 5α-dihydrotestosterone or estradiol. When injected together, 5α-dihydrotestosterone did not antagonize the effect of estradiol.

We conclude that one very high dose of 5α-dihydrotestosterone that stimulates leucine incorporation into proteins is also able to occupy the cytosol ER in mammary tumors and to translocate it in vivo into the nucleus. We suggest that a direct interaction of androgens with the ER located in mammary tumors is responsible, as in rat uteri, for the stimulating effect of very high doses of androgens. We cannot ascertain that the same mechanism is involved in the androgen-induced regression of the mammary tumors.

INTRODUCTION

It is generally agreed that estrogens via their receptors are involved in the induction and growth of breast cancer. The role of androgens is less clearly established. These hormones can stimulate growth of androgen-responsive mammary tumors such as the Shionogi 115 (1) and prevent growth of human breast cancer, for which the androgens give a 30% remission rate (12).

The mechanism by which androgens induce this mammary tumor regression is unknown, and several hypotheses have been proposed (13, 23) which deal either with an indirect effect of androgen on the hypothalamapituitary axis or with a direct effect on the mammary tumor itself. The possibility of androgens having an indirect effect through their aromatization into estrogens has also been considered; however, this is very unlikely since androgens such as DHT3 cannot be aromatized (11).

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1 This work was supported by the Institut National de la Sante et de la Recherche Medicale and the Fondation pour la Recherche Medicale.

2 To whom requests for reprints should be addressed.

3 The abbreviations used are: DHT, 5α-dihydrotestosterone; ER, estradiol receptor; DMBA, 7,12-dimethylbenz(a)anthracene; TET, 10 mM Tris, 1.5 mM; EDTA, 12 mM monothioglycerol HCl buffer (pH 7.4); DCC, dextran-coated charcoal; TCA, trichloroacetic acid; Re, cytosolic estrogen receptor; Rn, nuclear estrogen receptor.
oil as a control. In one case, a 2-day ovariectomized rat was anesthetized with Nembutal (20 mg/kg), and several biopsies were collected either before (time 0) or during the 5 hr following the injection of DHT or oil. The biopsy tissue was dissected free of fat and necrotic tissue and was used immediately or frozen in liquid nitrogen and stored for 1 to 3 days at -20°.

**Preparation of Extract.** The tumor tissue was washed in TET buffer, and homogenized in 10 volumes of TET buffer with a glass:glass Potter-Elvehjem homogenizer. The homogenate was centrifuged at 250,000 × g for 60 min, and the supernatant was defined as the cytosol. The pellet was homogenized in TET buffer plus 0.4 M KCl (pH 8.5), extracted 40 min at 2°, and then ultracentrifuged at the same speed. The KCl particulate extract obtained was shown to contain the majority of the extractable nuclear receptors (8).

**Estrogen Receptor Assays.** The ER sites of cytosol and particulate KCl extract were generally assayed after incubation with DCC (charcoal, 0.5%:dextran, 0.05%) for 2 hr at 2° with 2 saturating concentrations (5 and 10 nM) of [³H]estradiol and a 200-fold excess of diethylstilbestrol for nonspecific binding. The details and validity of this in vitro assay have already been shown in the DMBA mammary tumor (24). After DHT injection, the estrogen receptors were labeled in vitro by [³H]estradiol with an exchange technique previously tested on the rat uterine extracts (19). The free steroids were removed by incubation with DCC for 15 min at 2°. The extracts were then incubated in the presence of 10 nM [³H]estradiol plus 2 M diethylstilbestrol for 15 hr at 2°. The total ER sites (accessibles plus exchanged at 2°) of the extracts were then assayed in duplicate with DCC. All values were corrected for nonspecific binding determined by parallel incubation with 2 µM nonradioactive diethylstilbestrol.

**Competitive Experiments.** Nonradioactive androgens (5 µM) were incubated in tumor cytosol for 1 hr at 2°. Then [³H]estradiol (1 nM) was added for 5 min, and the association was stopped by adding nonradioactive estradiol (1 µM). The [³H]estradiol-receptor complexes were then assayed by DCC adsorption. After a 5-min incubation, the proportion of the nonspecific binding was negligible (1 to 3%) and was therefore not corrected.

**Sucrose Gradient Ultracentrifugation.** Aliquots of cytosol and nuclear extracts labeled with [³H]estradiol were analyzed in a 5 to 20% linear sucrose gradient at 206,000 × g for 13 to 14 hr at 2° in an SW 50 rotor with a Beckman L55B ultracentrifuge. Two-drop fractions were collected after puncturing the tube and were counted.

**In Vitro [³H]Leucine Incorporation.** Immediately after removal, the tumor tissue was cut into 1-cm pieces and rinsed in incubation medium. Two lots of 50 mg of the sliced tumor were incubated separately in a 20-ml flask containing 4 ml minimal essential medium/Earle's salt solution with 5 µCi/ml [³H]leucine (specific activity, 1 Ci/mmole). The incubation was carried out in a shaker-incubator at 37° under an O₂:CO₂ (95:5) atmosphere. Tumor pieces were then rinsed 3 times with 10 ml ice-cold TET buffer and homogenized in 2 ml of the same solution. Homogenates were centrifuged at 27,000 × g for 50 min at 2°. The soluble proteins in 2-aliquot fractions (200 µl) of the supernatant were precipitated with cold TCA and hydrolyzed in 0.1 N NaOH at 80° as previously described (4).

**Miscellaneous Techniques.** Protein concentrations were evaluated according to their absorption at 280 and 260 nm (9). The radioactivity was counted in a 5 ml scintillating counter (Intertechnique, Plaisir, 78, France) in 10 ml PPO (0.3%), POPOP (0.01%), toluene scintillation mixture with a 20 to 25% efficiency for tritiated steroids as evaluated by external standard. The hydrolyzed TCA-insoluble material containing [³H]leucine was counted in 5 ml Triton X-100 and 10 ml of the toluene scintillation mixture with a 20 to 22% efficiency.

**RESULTS**

**Binding Specificity of Androgens on the Estrogen Receptor.** Androgens have been shown to compete specifically with estradiol on the uterine ER (19). Similar experiments were done with the cytosol prepared from the DMBA rat mammary tumors and by use of the same "sensitized" competitive experiments in which the nonradioactive androgens were preincubated with cytosol before addition of [³H]estradiol for a short period of time (see "Materials and Methods"). This procedure allowed us to detect a significant inhibition of estradiol binding by weak affinity ligand, such as testosterone, which could not be shown by the classical competitive experiments performed at equilibrium.

Table 1 shows that the highest competitive efficiency was found for androgens containing 2 hydroxyl groups at the 3β and 17β positions such as Δ5-androstene-3β,17β-diol and 5α-androstane-3β,17β-diol. The removal of one of these groups decreased the affinity for ER, whereas 17α-estradiol and the C11 steroids, like progesterone, displayed no affinity. Dromostanolone propionate was completely inefficient to compete on ER contrary to the free

<table>
<thead>
<tr>
<th>Table 1 Binding specificity of androgens on the estrogen receptors in the DMBA-induced rat mammary tumors</th>
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<tbody>
<tr>
<td>Steroid†</td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td>None</td>
</tr>
<tr>
<td>Δ5-Androstene-3β,17β-diol</td>
</tr>
<tr>
<td>5α-Androstane-3β,17β-diol</td>
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<tr>
<td>5α-Dihydrotestosterone</td>
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<td>Dromostanolone</td>
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<td>Dihydroepiandrosterone</td>
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<tr>
<td>Testosterone</td>
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<tr>
<td>17α-Testosterone</td>
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<tr>
<td>5β-Dihydrotestosterone</td>
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<tr>
<td>Dromostanolone propionate</td>
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<tr>
<td>Progesterone</td>
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</tbody>
</table>

† Cytosol aliquots from mammary tumors collected 2 days after castration were preincubated for 1 hr with 5 µM of the indicated radiolabeled steroids and then for 5 min with 1 nM [³H]estradiol as described in "Materials and Methods."

² Results are expressed as percentage of bound [³H]estradiol measured without competing steroid.

³ Mean ± S.E. of the values determined from at least 3 different tumors.
dromostanolone, indicating that the 17β-hydroxyl must be free. We conclude that the binding specificity of estrogen receptors for androgens is similar in mammary tumors to that observed in uteri.

**In Vivo Nuclear Translocation of ER Induced by DHT.**

Twenty four hr after a bilateral ovariectomy, the estrogen receptors were assayed in the cytosol and in the KC1 particulate extract before and 2 to 5 hr after DHT treatment with the DCC exchange assay at 2° as described in “Materials and Methods.” We controlled the validity of this exchange technique on mammary tumor cytosol after in vitro occupation of ER sites by 5 μM DHT, 5α-androstane-3β,17β-diol, or 5 nM estradiol. As shown in Chart 1, after 15 hr incubation at 2° the bound DHT or 5α-androstane-3β,17β-diol could be totally displaced by [3H]estradiol, while the bound estradiol was only partially exchanged. The fact that we recovered the majority of the solubilizable (Rc plus Rn) ER sites after in vivo DHT injection in most of the tumors also validated this exchange procedure. As shown in Chart 2, the level of ER in the cytosol of biopsies removed 1 day following ovariectomy varied widely from 1 to 9 pmol/g of tumor (or 30 to 300 fmol/mg cytosol protein). The injection of 20 to 50 mg DHT in the rat induced a decrease of ER concentration in the cytosol and an increase in the particulate extract in 75 and 67% of the tumors, respectively. After DHT treatment, the average decrease of ER in the cytosol was 1.26 ± 0.47 (S.E.) pmol/g tumor (p < 0.02), and its average increase in the particulate extract was 0.54 ± 0.2 pmol/g tumor (p < 0.02) (Chart 3). In spite of a large variation according to the tumor, it generally appeared that the totality of the solubilizable ER (Rc plus Rn) was not recovered after DHT treatment. In these experiments, we did not specify whether this apparent loss of sites was due to some artifact or whether it was biologically significant. In Tumor 3 no translocation was observed because the Rc and Rn sites remained constant. However, the DHT-induced nuclear translocation of ER was significant in 92% of the tumors and constant in all tumors tested 2½ hr after DHT injection.

With DHT that had been purified further by 2 successive silica gel chromatographies as described in “Materials and Methods,” the nuclear translocation of ER was also demonstrated (Chart 2, ●). The time course of the DHT-induced nuclear translocation was followed with successive biopsies in one rat anesthetized with Nembutal and bearing 3 different tumors (Chart 4). The lack of effect of the anesthetic alone on the localization of ER already described by Nicholson et al. (16)
Androgen on Mammary Tumor Receptor

Chart 4. Time course of the ER nuclear translocation induced by DHT. Rc and Rn were assayed in biopsies taken from 3 separate tumors (Tumors 7, 8, and 9) developed in the same rat either before or at the indicated times following DHT injection. Results are expressed as a percentage of the cytosol receptor level of each tumor before DHT injection. Points, mean; bars, S.E.

was confirmed in a parallel experiment (not shown). DHT was able to decrease partially the Rc concentration and to increase the Rn concentration. A maximal ER translocation was observed 2 to 3 hr after DHT as already shown in the rat uterus (19), and the Rc sites appeared to be subsequently replenished. In fact, in 3 other tumors analyzed separately, a good recovery of the Rc binding sites was noticed 24 hr after DHT injection, suggesting an actual replenishment of the cytosol receptor (Table 2).

The nuclear estrogen-binding proteins present in the KCl particulate extract before and after DHT treatment were analyzed in a sucrose gradient after their in vitro labeling with [3H]estradiol (Chart 5). After DHT treatment and a subsequent exchange for 20 hr with [3H]estradiol, a well-defined saturable 3 to 4S binding peak was observed. On the other hand, in the absence of DHT (Chart 5, 20-hr control) or without the 20-hr exchange procedure (DHT 1 hr), the amount of 3 to 4S binding peak was much less important, which suggests that additional nuclear ER sites had been translocated to nuclei by DHT. The significance of the saturable binding peak observed after 1-hr (DHT) or 20-hr (control) labeling with [3H]estradiol was not specified. It could be due to accessible or very easily exchangeable ER sites.

The nature of the ligand responsible for the nuclear translocation of ER after administration of DHT was not specified. However, thin-layer chromatography of the tumor extract obtained 3 hr after injection of 50 mg [3H]DHT (specific activity, 0.6 mCi/mmol) showed that ~80% of the radioactivity migrated as nonmetabolized DHT, 10% as a 5α-androstanediol and 10% as 2 more polar compounds. The evaluated DHT uptake in the tumor (~1.7 μg/g tissue) gave an approximate final concentration of 0.4 μM in a cytosol of 2 mg protein per ml. This relatively low concentration of DHT as compared with the high dose injected may account for the weak in vivo ability of DHT to translocate ER in mammary tumors. These results indicated that DHT was able to translocate ER to the nucleus in 92% of the tumors studied. However, the degree of the nuclear translocation varied markedly according to the experiment and was more visible for the ER-rich tumors.

Effect of DHT on the Rate of [3H]Leucine Incorporation Into Soluble Proteins. The incorporation of [3H]leucine into soluble proteins was assayed after in vitro incubation of pieces of tumor collected either before or 24 hr after an in...
vivo injection of oil or 50 mg DHT (Chart 6). The in vitro 

[H]leucine incorporation into TCA-precipitable material was checked to be linear with time during a 2-hr incubation period. It was also homogeneous for different localizations in the same tumor since it varied less than 8% according to the tissue samples. However, a large variability was noticed from one tumor to another since the [H]leucine incorporated into protein varied between 2,000 and 15,000 cpm/mg protein per hr.

As shown in Table 3, a significant stimulation of 

[H]leucine incorporation into protein was induced by DHT in 60% of the treated tumors. In Tumors 7, 12, and 19 where the ER nuclear translocation induced by DHT was verified, the [H]leucine incorporation was also stimulated by DHT. In addition, the lack of response in some tumors seemed to be independent of the time following DHT injection (Tumor 20). A significant stimulation of [H]leucine incorporation was also obtained with estradiol (10 μg) in ~50% of the tumors. It is probable that the proportion of responsive tumors is underestimated. Actually, in Tumor 26, only the second injection appeared to be efficient. When the rats received both DHT and estradiol, a similar [H]leucine incorporation was observed as with estradiol alone, suggesting that DHT did not antagonize the estradiol effect. Actually, the means of the stimulation of leucine incorporation by DHT, estradiol, and DHT plus estradiol were 140 ± 16, 188 ± 35, and 203 ± 50%, respectively, and were not statistically different. Further experiments would be needed to ascertain an additive effect of estradiol and DHT. When rats were treated by oil in control experiments, the [H]leucine incorporation decreased from Days 2 to 3 after castration. This decrease, already described by others (6, 15) was probably due to ovarietomy and could be responsible for an underestimation of the stimulating effect of DHT and estradiol.

On the basis of the studies performed on the uterus (4), it is most likely that the observed increase of leucine incorporation into protein was mainly due to the stimulation of general protein synthesis. However, other possibilities were not excluded since the TCA-soluble [H]leucine was also slightly increased by DHT (not shown).

We concluded that a single high dose of DHT was able to stimulate leucine incorporation into tumoral proteins of castrated rats but was unable to prevent estradiol action on the same parameter.

**DISCUSSION**

When rats bearing DMBA-induced mammary tumors were treated by a high pharmacological dose of DHT, we observed 2 kinds of responses. On the one hand, the estrogen receptor sites are occupied in vivo by weak affinity ligand(s) and are subsequently transferred to the nucleus. On the other hand, the general protein synthesis is increased in approximately 60% of the tumors. The interaction of androgens with the ER sites has already been shown in the rat uterus (4, 19). Here, however, the degree of nuclear translocation varied markedly according to the tumor. The reason for this variation has not been specified. It could be due to a different degree of DHT uptake or metabolism or to different hormone responsiveness of the tumor. In addition, the proportion of ER transferred to the nucleus was not as high as that obtained with estradiol in the same type of tumors (20) or with DHT in rat uterus (19). Competitive experiments performed with the cytosol from mammary tumors indicate that the binding specificity of androgens for ER in the rat mammary tumor is similar to those observed in the rat uterus (19) and in human uterus and breast cancer (18). Recently, Davies et al. (2) independently reached the same conclusion on the rat mammary tumors. Dromostanolone propionate which is currently used in vivo to treat breast cancer was unable to compete with ER, contrary to the free steroid which is probably liberated in vivo by hydrolysis. The ER nuclear translocation observed in vivo after DHT injection is in agreement with the binding affinity of DHT for ER as determined in vitro and strongly suggests a direct interaction of androgens on the estrogen receptor under in vivo conditions. A decrease of the in vivo uptake of [H]estradiol had been suggested in mammary tumors after injection of up to 1 mg testosterone (14) and in rat uterus after injection of 3 mg DHT (M. Garcia, unpublished observations). In our case, the nature of the androgen responsible for the ER nuclear translocation, either untransformed DHT and/or DHT metabolite(s), cannot be ascertained, since in addition to a majority of DHT other compounds like androstaneol were extracted from the tumor. The responsibility of a high-affinity metabolite of DHT present in small amounts could not be excluded by thin-layer chromatography; however, it is unlikely on the basis of the rapid exchangeability of ER sites obtained at 2°. Similarly, the involvement of a minute amount of contaminating estrogens in the DHT preparations is very unlikely for the following reasons. (a) As shown in Chart 1 after estradiol binding, it would not be possible to exchange totally the sites at 2°; if so, the recovery of ER sites would have been very low. The same difference in exchangeability was also found after in vivo occupation of ER sites by estradiol or DHT (not shown). (b) A contamination by estrogens would not be in agreement with the binding stereospecificity of the different androgens shown in Table 1. (c) We have recently demonstrated the direct binding of
Table 3

In vivo effects of DHT and estradiol on the in vitro [3H]leucine incorporation into soluble proteins

Two pieces of the same tumor of a 2-day ovariectomized rat were removed either before or at the indicated time following the injection of oil, DHT (50 mg), estradiol (10 μg), or DHT (50 mg) plus estradiol (10 μg). The tissue samples were then immediately incubated at 37° in minimal essential medium containing [3H]leucine for 1 hr, and the incorporation of [3H]leucine into protein was assayed as described in "Materials and Methods." The [3H]leucine incorporation in the hormone-treated tumor is expressed as the percentage of the values obtained in the biopsy of the same tumor collected before treatment. For each biopsy 2 to 4 separate incubations were performed, and the mean ± S.D. was taken as the 100% value. p values express the statistical significance of the variation of [3H]leucine incorporation observed in the treated tumor as compared to the biopsy.

<table>
<thead>
<tr>
<th>Tumor n°</th>
<th>Time following injection (hr)</th>
<th>Control biopsy</th>
<th>Treated tumor</th>
<th>p</th>
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<tr>
<td>7</td>
<td>4</td>
<td>100 ± 2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>171 ± 5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>12</td>
<td>4</td>
<td>100 ± 2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>129 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>19</td>
<td>4</td>
<td>100 ± 6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>170 ± 9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>20</td>
<td>4</td>
<td>100 ± 17</td>
<td>75 ± 9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NS&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>11</td>
<td>24</td>
<td>100 ± 17</td>
<td>76 ± 9&lt;sup&gt;a&lt;/sup&gt;</td>
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</tr>
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<td>21</td>
<td>17</td>
<td>100 ± 14</td>
<td>132 ± 14&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>22</td>
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<td>100 ± 4&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>17</td>
<td>24</td>
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<td>23</td>
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<td>82 ± 8&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Oil</td>
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<td>100 ± 15</td>
<td>61 ± 2&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>24</td>
<td>4</td>
<td>100 ± 13</td>
<td>68 ± 23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NS</td>
</tr>
<tr>
<td>Estradiol (10 μg)</td>
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<td>100 ± 10</td>
<td>224 ± 28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.05</td>
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<tr>
<td>26&lt;sup&gt;c&lt;/sup&gt;</td>
<td>24</td>
<td>100 ± 7</td>
<td>57 ± 7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.05</td>
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<tr>
<td>26&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>100 ± 33</td>
<td>208 ± 32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NS</td>
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<td>27&lt;sup&gt;e&lt;/sup&gt;</td>
<td>24</td>
<td>100 ± 6</td>
<td>225 ± 19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.05</td>
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<tr>
<td>28&lt;sup&gt;f&lt;/sup&gt;</td>
<td>24</td>
<td>100 ± 12</td>
<td>283 ± 16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.01</td>
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<td>29&lt;sup&gt;f&lt;/sup&gt;</td>
<td>24</td>
<td>100 ± 4</td>
<td>155 ± 21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NS</td>
</tr>
<tr>
<td>Estradiol (10 μg) + DHT (50 mg)</td>
<td>24</td>
<td>100 ± 12</td>
<td>253 ± 16&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>30</td>
<td>24</td>
<td>100 ± 16</td>
<td>333 ± 16&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>33&lt;sup&gt;e&lt;/sup&gt;</td>
<td>e</td>
<td>100 ± 10</td>
<td>247 ± 10&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>34&lt;sup&gt;e&lt;/sup&gt;</td>
<td>e</td>
<td>100 ± 5</td>
<td>147 ± 5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.02</td>
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</table>

<sup>a</sup> Mean ± S.D. for 2 to 4 separate incubations.
<sup>b</sup> NS, not significant.
<sup>c</sup> This experiment was performed 6 days after castration.
<sup>d</sup>,<sup>e</sup> A second injection of estradiol (d) or of estradiol plus DHT (e) was administered 72 hr after a first injection of estradiol (10 μg). The effect was observed 24 hr after the second injection.

androgens to ER with tritiated androgens. Finally, the DHT used had been crystallized twice. In control experiments, DHT purified by 2 successive silica gel chromatographies displayed the same nuclear transfer ability as did ER (Chart 2, e). We have checked by adding [3H]estradiol to DHT that the contamination of DHT by estradiol or estriol was less than 10<sup>-4</sup> after these purification steps.

The metabolic response to DHT reported in this paper is the stimulation of leucine incorporation into protein. This effect agrees with the increase of tumor size also observed in some hormone-dependent DMBA mammary tumors after injection of DHT (50 mg) daily for 5 days (unpublished observations) or of testosterone propionate (T5). It is probable that the stimulation of protein synthesis observed after DHT administration is due to the ER nuclear translocation. In addition to ER the progesterone receptor and the androgen receptor, which both bind DHT, could also be responsible a priori for the stimulating effect of DHT observed. The androgen receptor sites are present in the DMBA tumor, although in small concentrations (0 to 30 fmol/mg protein) (F. Vignon and M. Garcia, unpublished observations). However, the growth of the DMBA rat mammary tumors was never stimulated by doses of androgens that are able to occupy the androgen receptor sites but not the progesterone or the estrogen receptor sites. An interaction of androgens on the progesterone receptor cannot be excluded here since progesterone has been shown to stimulate DNA synthesis in rat mammary tumors (17).

<sup>4</sup> M. Garcia and H. Rochefort, manuscript in preparation.
However, evidence suggests that the ER-androgen complexes are in fact responsible for the stimulating effect of very high doses of DHT. The time- and dose-response studies of the ER nuclear translocation and of the [3H]leucine incorporation are similar to those observed in the rat uterus (4). Moreover, preliminary experiments have shown that DHT as estradiol induces the progesterone receptor in these DMBA rat tumors. A similar induction of the progesterone receptor has been shown in the MCF, human breast cancer cell line (26). Finally, as in the case of estrogens, not all tumors were responsive to DHT. Actually, in this study, only 60% of the tumors were stimulated by DHT for leucine incorporation, while most of the tumors were responsive to ER nuclear translocation. However, we cannot now ascertain whether the same receptor mechanism is involved in the regression of mammary tumors provoked by lower doses of androgens.

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tained 24 hr after the last injection (25). Preliminary experi-

ments indicated to us that these low doses of DHT were also insufficient 2 to 3 hr after DHT injection, which are the optimal conditions for evaluation of the ER nuclear trans-

location (unpublished observations). Conversely, at higher doses giving the nuclear translocation of ER as reported previously, tumor growth was stimulated rather than in-

hibited. For explanation of these discrepancies, we propose that DHT at very high doses is an agonist and that the DHT-

ER interaction reported in this paper is responsible for the stimulation of protein synthesis and tumor growth subse-

quently observed. For lower doses of androgens that are known to induce tumor regression but do not seem to provoke ER nuclear translocation, other mechanisms must be invoked. In this case, DHT could still be acting via ER as an estrogen antagonist by binding to Rc without activating it, or the effect of DHT could be mediated by a completely different receptor mechanism.

From a practical point of view, the effect of androgen on the estrogen receptors of tumors might ameliorate our understanding of the mechanism of action of additive androgen therapy and/or suppressive adrenalectomy on breast cancer. A direct interaction of endogenous andro-

gens with ER could also be involved in the induction and/or promotion of mammary cancer since the concentrations of adrenal androgens such as Δ5-androstenediol both in fe-

male plasma (2 to 3 nm) (18) and in human breast cancer (10 to 200 ng/g tumor) (10) appear sufficient to interact efficiently with ER if one assumes the relative high affinity of Δ5-androstenediol (Kd = 4.5 nm).4

In conclusion, a very high dose of DHT which stimulates the cytosol ER in mammary tumors and to translocate it in vivo into the nucleus. We hypothesize that this androgen-

induced translocation of ER is responsible for the stimulat-

ing effect of very high doses of androgens on protein synthesis and tumor growth. However, we cannot now ascertain whether the same receptor mechanism is involved in the regression of mammary tumors provoked by lower doses of androgens.
Androgen Effects Mediated by Estrogen Receptor in 7,12-Dimethylbenz( a)anthracene-induced Rat Mammary Tumors

Marcel Garcia and Henri Rochefort


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