Effect of Ovariectomy on Hormone Receptors and Growth of 
N-Nitrosomethylurea-induced Mammary Tumors in the Rat

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ABSTRACT

Estrogen receptor(s) (ER), progesterone receptor(s) (PGR), androgen receptor(s) (ANR), and prolactin receptor(s) (PRLR) were measured in N-nitrosomethylurea-induced mammary tumors in intact female Sprague-Dawley rats and in rats 9 days after ovariectomy. Following ovariectomy, 12 of 15 tumors regressed to 47.7 ± 5.5% of the original size (hormone-dependent tumors), while the remaining three had arrest of growth reaching 88.8 ± 7.3% of their original sizes.

Cytosol ER level was 50.3 ± 6.6 fmol/mg protein in tumors of intact rats and was significantly lower (25.6 ± 8.3 fmol/mg, p < 0.025) in the ovariectomized group. PGR was abundantly present in ten of 13 tumors of intact rats (mean, 144.5 ± 46.8) but was undetectable in five of six hormone-dependent tumors after ovariectomy (p < 0.01). ANR was detectable at low levels in only four of 13 tumors of intact rats but in none of six hormone-dependent tumors after ovariectomy. PRLR was not significantly different in tumors of intact and ovariectomized rats (20.6 ± 2.4 and 15.6 ± 1.8 fmol/mg, respectively). In three tumors that had arrest of growth after ovariectomy, the levels of ER, PGR, ANR, and PRLR were not significantly different from those of the hormone-dependent tumors.

We conclude that the majority of N-nitrosomethylurea-induced rat mammary tumors are hormone dependent. ER, PGR, and PRLR were abundantly present in the majority of these tumors, while ANR was present in only four of 13 tumors. The levels of ER and PGR were significantly lower following ovariectomy, while PRLR was not significantly changed.

INTRODUCTION

NMU3-induced mammary tumors in rats have been noted to be hormone dependent (2, 11–13). Castration before or immediately after the administration of NMU significantly decreases the number of tumors induced (2). Tumor regression has been obtained with ovariectomy, tamoxifen, or luteinizing hormone-releasing hormone analog administration (11–13). Of interest is a recent preliminary observation that the growth of these tumors can be reactivated with estradiol administration following luteinizing hormone-releasing hormone analog-induced regression but not with perphenazine treatment after an ovariectomy-induced tumor regression (11). Since perphenazine stimulates endogenous PRL secretion, this observation suggests that this tumor model may be estradiol rather than PRL dependent and thus may resemble human breast cancer (9) more than the widely used 7,12-dimethylbenz(a)anthracene-induced mammary tumor which is PRL dependent (1).

A role of PRL, however, in tumorigenesis has been described for the NMU-induced mammary tumors. The administration of a PRL inhibitor, bromocryptine, immediately after NMU administration significantly reduces the number of tumors recovered (16).

Receptors for estrogen (11, 13), progesterone (5, 6), glucocorticoid (5), and prolactin (15) have been described in preliminary reports in NMU-induced mammary tumors. In the present experiment, we measured ER, PGR, and PRLR as well as ANR in the tumors of intact and castrated rats.

MATERIALS AND METHODS

Animals and Tumors. Tumors were induced by one of us (P. G.) in 50-day-old female Sprague-Dawley rats as previously described (2). Bilateral ovariectomy was performed through dorsal midline incision under light ether anesthesia.

Mammary tumors were measured with a caliper twice weekly. Tumor sizes were expressed in sq cm and derived from the product of the 2 major axes. Thirteen tumors were removed from intact rats and served as control. Fifteen tumors were observed following ovariectomy for 9 days. At that time, only 9 were large enough for receptor assay. Tumors were excised, immediately frozen in liquid nitrogen, and stored at −60° until the assays were performed.

Tumors were defined as hormone dependent if they decreased to at least ≤65% of the original size during the first 9 days after ovariectomy.

Materials. NMU wet in 3% acetic acid was obtained from K & K Laboratories, Plainview, N. Y. 17β-[2,4,6,7-3H]Estradiol (specific activity, 108 Ci/mmol) was obtained from Amersham/Searle Corp., Arlington Heights, Ill. R5020 (specific activity, 87 Ci/mmol) and the corresponding unlabeled hormone were obtained from New England Nuclear, Boston, Mass. [3H]DHT (specific activity, 123 Ci/mmol) and the unlabeled hormone were obtained from New England Nuclear. Ovine PRL was a gift from National Institute of Arthritis, Metabolism, and Digestive Diseases. 17β-Estradiol was obtained from Sigma Chemical Co., St. Louis, Mo.

Hormone Receptor Measurement. ER in the cytosol was measured using the dextran-coated charcoal method (10). Briefly, the 100,000 x g cytosol fraction was incubated for 16 hr at 4° with serial dilutions of [3H]estradiol (from 0.06 to 0.8 nm). The free radioactivity was removed by adding dextran-coated charcoal with subsequent shaking for 30 min and spinning at 800 x g for 10 min. Aliquots of the supernatant...
were then transferred to scintillation vials and counted in a β counter. Scatchard plot analysis (14) was used to determine the number of binding sites expressed as fmol/mg cytosol protein.

PGR was measured in the cytosol fraction using the synthetic progestin R5020 and the sucrose density ultracentrifugation technique (4). In brief, the 100,000 × g cytosol fraction was incubated for 4 hr at 4°C with labeled R5020 (20 nM) in the presence or absence of 200-fold excess unlabeled R5020. After removing the free radioactivity with dextran-coated charcoal, aliquots of bound radioactivity (both specific and nonspecific) were layered on top of a 5 to 20% sucrose continuous gradient and spun for 16 hr at 55,000 rpm. Subsequently, the bottoms of the centrifugation tubes were punctured, and the bound radioactivity was collected in fractions of 6 drops each. The number of specific binding sites was calculated by subtracting the nonspecific binding from the total binding and was expressed as fmol/mg cytosol protein.

ANR was measured in the cytosol fraction using the dextran-coated charcoal technique (3) with minor modifications. In brief, the tissue was pulverized in the Thermovac and then homogenized in buffer (50 mM Tris-HCl-0.1 mM EDTA-0.5 mM 2-mercaptoethanol, pH 7.4). The homogenate was then centrifuged at 100,000 × g for 26 min to obtain the cytosol fraction. Aliquots of the diluted cytosol (1 to 2 mg/ml) were incubated for 2 hr at 4°C with increasing amounts of labeled DHT (0.07 nM —> 2 nM) in the presence or absence of 100-fold excess unlabeled DHT. The free radioactivity was removed by adding dextran-coated charcoal with subsequent shaking for 30 min and spinning at 800 × g for 10 min. Aliquots of the supernatant were then counted in a β counter. Scatchard plot analysis (14) was used to determine the number of binding sites expressed as fmol/mg cytosol protein.

PRLR determination was carried out using a method published recently (8). Briefly, the 5,000 × g membrane fraction was incubated for 16 hr at room temperature with 125I-labeled PRL (100,000 cpm) in the presence or absence of serial dilutions of unlabeled PRL. Subsequently, 3 ml of chilled buffer were added, the tubes were centrifuged, the supernatant was decanted, and the pellets were counted in a γ counter. The number of specific binding sites was calculated by Scatchard analysis (14) and expressed as fmol/mg protein.

RESULTS

Tumor Growth. Twelve of 15 tumors regressed to ≤ 65% of their original size (hormone dependent) after ovariectomy, reaching 47.7 ± 5.5% of the original sizes in 9 days. Three additional tumors had arrest of growth reaching 88.8 ± 7.3% of the original size during the same period following ovariectomy. No autonomous tumors were encountered in this experiment.

Hormone Receptors. Table 1 summarizes the data on ER, PGR, ANR, and PRLR in all tumors studied from intact and ovariectomized rats. ER was abundantly present in all mammary tumors of intact and ovariectomized rats; however, it was significantly lower in the ovariectomized group.

PGR was present in 10 of 13 mammary tumors and undetectable (<3 fmol/mg cytosol protein) in 3 other tumors of intact rats. Five of the 10 positive tumors showed both an 8S and 4S peak, whereas 5 manifested only a 4S peak. Five of 6 hormone-dependent mammary tumors of ovariectomized rats had undetectable PGR while one had a low positive value.

ANR was detected (≤4 fmol/mg cytosol protein) in only 4 of 13 tumors of intact rats and in none of 6 hormone-dependent tumors after ovariectomy. PRLR was abundantly present in all tumors of intact and ovariectomized rats at similar levels.

ER, PGR, ANR, and PRLR in mammary tumors were not significantly different in the hormone-dependent tumors versus those that had arrest of growth following ovariectomy.

DISCUSSION

Our study confirms the presence of ER, PGR, and PRLR in the NMU mammary tumors as described previously in the literature (5, 6, 11, 13, 15). The receptor characteristics tested, namely the Kd for ER and PRLR and the sucrose density gradient profile for PGR, are in agreement with those reported by others (6, 15), although Lewko et al. (5) observed the presence of only a 4S peak for PGR. We also observed low levels of ANR in 4 of 13 tumors of intact rats. The linear Scatchard plots obtained and the Kd indicate that these are a single class of high-affinity binding sites. In the present experiment, we did not test the ability of other unlabeled hormones to displace labeled DHT.

Following ovariectomy, we observed a significant fall in ER and virtual disappearance of PGR. None of the tumors of ovariectomized rats contained ANR. Lindsey et al. (7) and Turcot-Lemay and Kelly (15) have also reported, although only in abstract form, a decline in ER following ovariectomy. Somewhat at variance with our results, Wittliff et al. (17) still found large amounts of PGR in NMU mammary tumors 7 to 10 days after ovariectomy, whereas in our experience, 9 days after ovariectomy, PGR was virtually absent.

Table 1

<table>
<thead>
<tr>
<th>ER's, PGR's, ANR's, and PRLR's in NMU-induced mammary tumors</th>
<th>Intact rats</th>
<th>Ovariectomized rats</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>fmol/mg</td>
<td>Kd (× 10^-10 M)</td>
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<tr>
<td>ER</td>
<td>50.3 ± 6.68 (13)</td>
<td>0.53 ± 0.05 (13)</td>
</tr>
<tr>
<td>PGR</td>
<td>144.5 ± 46.8 (13)</td>
<td>1.4 ± 1.4 (6)</td>
</tr>
<tr>
<td>ANR</td>
<td>4.1 ± 1.9 (13)</td>
<td>Undetectable (6)</td>
</tr>
<tr>
<td>PRLR</td>
<td>20.6 ± 2.4 (13)</td>
<td>16.6 ± 1.8 (6)</td>
</tr>
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<tr>
<th>Hormone dependent</th>
<th>fmol/mg</th>
<th>Kd (× 10^-10 M)</th>
<th>fmol/mg</th>
<th>Kd (× 10^-10 M)</th>
</tr>
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<tbody>
<tr>
<td></td>
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<tr>
<td>ER</td>
<td>25.6 (6)</td>
<td>0.67 ± 0.19 (6)</td>
<td>32.9 ± 6.3 (3)</td>
<td>0.77 ± 0.3 (3)</td>
</tr>
<tr>
<td>PGR</td>
<td>1.4 (6)</td>
<td>Undetectable (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANR</td>
<td>6.9 ± 1.86 (4)</td>
<td>17.6 ± 2.7 (3)</td>
<td>1.18 ± 0.22 (3)</td>
<td></td>
</tr>
</tbody>
</table>
It is of interest that we observed no difference in PRLR content of tumors of intact and ovariectomized rats. This is at variance with the findings by Turcot-Lemay et al. (15) who apparently found a decline in PRLR after ovariectomy. These authors, however, do not give any data in this regard in their abstract to allow a comparison of their findings with ours.

Several biological characteristics of NMU-induced mammary tumors, such as the ability to metastasize and induce hypercalcemia (2), have indicated that these tumors may resemble human breast carcinoma. Recent evidence strongly suggests that hormone-dependent human breast carcinoma is primarily estradiol dependent as shown by significant palliation obtained by antiestrogens in patients who underwent complete surgical hypophysectomy (9). If the NMU-induced mammary tumors are confirmed to be estradiol dependent, they would then be a more suitable experimental model than the widely used 7,12-dimethylbenz(a)anthracene-induced mammary tumors which are PRL dependent (1).

ACKNOWLEDGMENTS

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REFERENCES

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