Inhibition of Mammary Tumor Growth by Dexamethasone in Rats in the Presence of High Serum Prolactin Levels

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ABSTRACT

Female Sprague-Dawley rats with established 7,12-dimethylbenz(a)anthracene-induced mammary tumors were given daily s.c. injections of 50 μg dexamethasone per rat, 0.5 mg haloperidol per kg, or both for 3 weeks. Control rats received the injection vehicles only. Mammary tumor growth was measured at weekly intervals for 21 days, and blood was collected on Days 10 and 21 of treatment for assay of prolactin. Dexamethasone produced significant regression of mammary tumors and reduced serum prolactin levels, whereas haloperidol significantly increased mammary tumor growth and greatly elevated serum prolactin levels. When dexamethasone and haloperidol were injected together, there was significant regression of mammary tumors despite markedly elevated serum prolactin levels. No significant differences in specific prolactin binding to membrane preparations of mammary tumors from these animals were observed in any treatment group. These results indicate that dexamethasone, a synthetic glucocorticoid, can directly inhibit mammary tumor growth in the presence of elevated serum prolactin levels produced by haloperidol, and this inhibition is not due to a reduction of prolactin binding sites in the tumor tissue.

INTRODUCTION

Mammary tumors regress during postpartum lactation in rats (4, 11–13) and in women (14) even though serum prolactin levels are elevated above nonlactating levels (1). Prolactin has been shown to stimulate mammary tumor growth in rats (15, 20), and adrenal glucocorticoid hormones can inhibit mammary tumor growth (3, 8). We found that, when the adrenals were removed during postpartum lactation in rats, mammary tumors grew as well as or better than they did in nonlactating rats, indicating that the adrenals were responsible for inhibition of tumor growth during lactation (2). We also observed that in nonlactating rats adrenalectomy resulted in stimulation of mammary tumor growth and elevation of serum prolactin levels, whereas cortisol administration inhibited tumor growth and reduced serum prolactin levels (3). In view of these findings, it was of interest to determine whether administration of a potent synthetic glucocorticoid hormone, dexamethasone, could inhibit carcinogen-induced mammary cancer growth in nonlactating rats in the presence of elevated serum prolactin pro-

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Tumor Induction and Drug Treatment. Virgin female Sprague-Dawley rats (Harlan Research Animals, Indianapolis, Ind.), 55 days old, were given a single i.v. injection of 1 ml lipid emulsion containing 5 mg of DMBAA (kindly provided by Dr. P. Schurr, Upjohn Co., Kalamazoo, Mich.). The rats were housed in plastic cages in a temperature- and light-controlled room (24 ± 0.5°; 14 hr light and 10 hr dark) and fed rat chow (Ralston-Purina Co., St. Louis, Mo.) and water ad libitum. Approximately 8 weeks after DMBA administration, when each rat had at least one tumor measuring more than 1 cm in diameter, the rats were divided into groups. Group 1, controls, received daily 0.1-ml s.c. injections of each vehicle (0.3% ethanol and 0.9% NaCl solution). Group 2 was given a daily s.c. injection of 50 μg dexamethasone suspended in 0.1 ml 0.3% ethanol and a second daily injection of 0.1 ml 0.9% NaCl solution vehicle. Group 3 received a daily s.c. injection of haloperidol (0.5 mg/kg) suspended in 0.1 ml 0.9% NaCl solution and a second injection of 0.1 ml 0.3% ethanol vehicle. Group 4 received daily s.c. injections of 50 μg dexamethasone in 0.1 ml 0.3% ethanol and a daily s.c. dose of haloperidol (0.5 mg/kg) in 0.1 ml 0.9% NaCl solution. Thus, each rat received 2 daily injections in a total volume of 0.2 ml.

Tumor Measurements. Tumor measurements and body weights were recorded at weekly intervals after initiation of drug treatment for a 3-week period. Average tumor diameter for each palpable tumor was determined by using the mean of the 2 largest perpendicular diameters as measured with vernier calipers. Tumor growth was expressed as the percentage of change in average tumor diameter when compared with initial measurements made prior to initiation of drug treatment.

Blood Collection and Hormone Assays. Blood was collected under light ether anesthesia by orbital sinus puncture 10 days after the onset of drug treatment and by decapitation at the termination of the experiment (Day 21). At both periods, blood was collected between 10 and 11 a.m., 1 hr after the daily injection of the drug or vehicle. Serum was separated by centrifugation and stored at −20° until assayed for prolactin by a standard radioimmunoassay method (16). The prolactin antibody was kindly provided by C. L. Chen, College of Veterinary Medicine, University of Florida, Gainesville, Fla.

Tissue Preparation for Prolactin Receptor Assay. The method used to assay prolactin receptors in mammary tumor tissue has been described by us previously (9). Rats were killed by decapitation at the end of the experiment, and all

* The abbreviation used is: DMBA, 7,12-dimethylbenz(a)anthracene.
identifiable mammary tumors were immediately excised, wrapped in tinfoil, and frozen on dry ice. Tissues were stored at —50°C until assayed for prolactin-binding activity. All mammary tumors were homogenized in 0.3 M sucrose for 45 sec in a Waring Blender with a special microcup attachment.

The homogenate was centrifuged at 11,000 rpm for 20 min, and the pellet was discarded. The supernatant was centrifuged at 40,000 rpm for 60 min to obtain the particulate membrane pellet. This pellet was then resuspended in a Tris buffer (0.025 M Tris, pH 7.6, and 10 mM CaCl₂). Protein concentration for each membrane preparation was determined by the method of Lowry et al. (10). All samples were then diluted to uniformity with the Tris buffer so that 500 μg of membrane protein were present in 100 μl of membrane preparation.

Ovine prolactin was iodinated by the lactoperoxidase method of Thorell and Johansson (19). 125I-labeled prolactin was diluted in Tris buffer to give approximately 70,000 cpm/100 μl. Individual tumor samples were assayed in quadruplicate. Total ovine prolactin binding was determined in tubes containing 100 μl of 125I-labeled prolactin, 300 μl of membrane preparation containing 1.5 mg protein, and 100 μl of Tris buffer. Parallel incubations to determine nonspecific ovine prolactin binding were performed using the same reactants, except 100 μl of excess unlabeled ovine prolactin (1 μg/100 μl) replaced the 100 μl of Tris buffer diluent. In all tubes, the total incubation volume was 0.5 ml. All assay tubes were incubated for 2 days at 4°C, after which 3 ml Tris buffer were added to terminate the incubation, and the tubes were centrifuged at 1600 rpm for 30 min. The resulting pellets were counted for 1 min in a Nuclear-Chicago gamma counter. Specific binding was determined for each sample by subtracting the cpm bound in the presence of unlabeled ovine prolactin (i.e., nonspecific binding cpm) from the cpm bound in the absence of ovine prolactin (i.e., total binding cpm) and was expressed as a percentage of total radioactive label used in each incubation.

Statistical differences between treatment groups were determined by analysis of variance and then by the Student-Newman-Keuls statistical test. A difference of p < 0.05 was considered to be significant.

RESULTS

Table 1 and Chart 1 show the effects of dexamethasone and haloperidol on mammary tumor growth expressed as the actual change and the percentage of change in average tumor diameter. Dexamethasone, whether administered alone or together with haloperidol, produced a significant reduction in mammary tumor diameter. Dexamethasone alone decreased average tumor size about 40% as compared with controls 1 week after treatment (p < 0.05), about 50% 2 weeks after treatment (p < 0.01), and about 60% by the end of the 21-day treatment (p < 0.01). When dexamethasone was given with haloperidol, tumor size was significantly reduced by nearly 40% by the end of the treatment period, as compared to pretreatment values. The combined treatment resulted in about an 80% reduction in tumor size as compared with controls (p < 0.01). Haloperidol alone increased average tumor diameter by about 80% when compared with initial size, whereas an increase of only about 40% was present in the vehicle-injected controls.

Table 2 shows the effect of dexamethasone and haloperidol on average tumor number per rat. Rats treated with dexamethasone alone or together with haloperidol showed a decrease in average tumor number. However, these decreases were not statistically significant when compared with controls. Rats given haloperidol alone showed significant increases in average tumor number by 1 to 3 weeks as compared with control values. Table 2 also shows the number of tumors that completely regressed and were no longer palpable by the end of the treatment period. Administration of dexamethasone alone

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rats</th>
<th>No. of initial tumors</th>
<th>Av. tumor diameter (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle controls</td>
<td>8</td>
<td>18</td>
<td>Wk 0: 1.2 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dexamethasone (50 μg/rat/day)</td>
<td>8</td>
<td>22</td>
<td>Wk 0: 1.1 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Haloperidol (0.5 mg/kg/day)</td>
<td>8</td>
<td>21</td>
<td>Wk 0: 1.2 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dexamethasone + haloperidol</td>
<td>8</td>
<td>22</td>
<td>Wk 0: 1.2 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean ± S.E.
<sup>b</sup> p < 0.05 as compared with initial pretreatment values.
<sup>c</sup> p < 0.05 as compared with controls.
<sup>d</sup> p < 0.01 as compared with controls.

Chart 1. Effects of dexamethasone (DEX; 50 μg/rat/day), haloperidol (HAL; 0.5 mg/kg/day), and dexamethasone administered with haloperidol (DEX + HAL) on the percentage of change in average tumor diameter. * p < 0.05; ** p < 0.01 (treatment versus control).
decapitation. Hence, there are differences in serum prolactin values due to the different methods used.

Blood was collected at 10 days by orbital sinus puncture and at 21 days by decapitation. Hence, there are differences in serum prolactin values due to the two different methods used.

Haloperidol (0.5 mg/kg/day) resulted in complete disappearance of 12 of the original 22 mammary tumors. When dexamethasone was administered with haloperidol, 7 of 22 tumors disappeared. In the control and haloperidol-treated groups, none of the initial tumors regressed by the end of the experiment.

The effects of dexamethasone and haloperidol treatment on serum prolactin levels at both periods when compared with controls. However, only at Day 10 was this decrease significant (p < 0.05). Haloperidol administered alone or with dexamethasone significantly (p < 0.01) elevated serum prolactin above control levels at both periods. Furthermore, when dexamethasone was administered with haloperidol, serum prolactin levels were elevated above those produced by haloperidol alone. The differences in serum prolactin values within any treatment group may be explained by the 2 different methods of blood collection used at 10 days and at the end of the experiment.

The regression of mammary tumors induced by dexamethasone in the present study cannot be attributed to a reduction in body weight, since there were no significant changes in body weight. The disappearance of some of the mammary tumors in the rats given dexamethasone or dexamethasone and haloperidol probably reflects the direct inhibitory action of dexamethasone on the mammary tumor cells. An explanation for the greater rise in serum prolactin produced by the combination of haloperidol and dexamethasone as compared to that produced by haloperidol alone is not readily apparent at present.

REFERENCES


**Table 2**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rats</th>
<th>No. of initial tumors</th>
<th>No. of tumors/rat</th>
<th>No. of complete regressions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle controls</td>
<td>8</td>
<td>18</td>
<td>2.5 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0</td>
</tr>
<tr>
<td>Dexamethasone (50 µg/rat/day)</td>
<td>8</td>
<td>22</td>
<td>2.8 ± 0.5</td>
<td>12</td>
</tr>
<tr>
<td>Haloperidol (0.5 mg/kg/day)</td>
<td>8</td>
<td>21</td>
<td>2.6 ± 0.5</td>
<td>2</td>
</tr>
<tr>
<td>Dexamethasone + haloperidol</td>
<td>8</td>
<td>22</td>
<td>2.8 ± 0.7</td>
<td>7</td>
</tr>
</tbody>
</table>

<sup>a</sup> Total number of tumors at beginning of drug treatment.
<sup>b</sup> Mean ± S.E.
<sup>c</sup> p < 0.05 as compared with pretreatment values.
<sup>d</sup> p < 0.05 as compared with controls.

**DISCUSSION**

The present study shows that dexamethasone can produce regression of DMBA-induced mammary tumors in rats, regardless of whether serum prolactin levels are decreased by dexamethasone alone or increased when given together with haloperidol. Therefore, the ability of adrenocortical steroids to inhibit growth of mammary tumors in rats is not primarily dependent on the level of serum prolactin. Others have shown that dexamethasone can reduce (7, 18) and that haloperidol can increase (5) serum prolactin concentrations in rats. The primary mechanism by which dexamethasone inhibits mammary tumor growth apparently is via a direct action on the tumor tissue. The recent observation that dexamethasone can inhibit the growth of human breast cancer cells in vitro (17) supports this view. Dexamethasone did not reduce the number of specific prolactin receptors in the mammary tumors of our rats, indicating that this is not its mode of action. Dexamethasone may decrease the number of receptors for other hormones, such as estrogen, which is necessary in addition to prolactin for maintenance of mammary tumor growth in rats (15). Dexamethasone may act by inhibiting protein synthesis in the mammary tumor tissue, since such an effect on body tissues is well established for glucocorticoid hormones (6).

The present findings appear to be similar to our observations in postpartum lactating rats, in which regression of mammary tumors occurred in the presence of a presumed increase in adrenocortical secretion and elevated serum prolactin levels (2). Adrenalectomy of these rats resulted in resumption of mammary tumor growth equal to or surpassing that of nonlactating rats.

The regression of mammary tumors induced by dexamethasone in the present study cannot be attributed to a reduction in body weight, since there were no significant changes in body weight. The disappearance of some of the mammary tumors in the rats given dexamethasone or dexamethasone and haloperidol probably reflects the direct inhibitory action of dexamethasone on the mammary tumor cells. An explanation for the greater rise in serum prolactin produced by the combination of haloperidol and dexamethasone as compared to that produced by haloperidol alone is not readily apparent at present.

**Table 3**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rats</th>
<th>Serum prolactin (ng/ml)</th>
<th>Day 10&lt;sup&gt;4&lt;/sup&gt;</th>
<th>Day 21&lt;sup&gt;4&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle controls</td>
<td>8</td>
<td>68.8 ± 10.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.1 ± 4.2</td>
<td></td>
</tr>
<tr>
<td>Dexamethasone (50 µg/rat/day)</td>
<td>8</td>
<td>30.4 ± 3.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.1 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>Haloperidol (0.5 mg/kg/day)</td>
<td>8</td>
<td>208.2 ± 26.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>80.2 ± 10.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Dexamethasone + haloperidol</td>
<td>8</td>
<td>303.9 ± 55.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>211.7 ± 33.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>4</sup> Days after initiation of treatment.
<sup>b</sup> Mean ± S.E.
<sup>c</sup> p < 0.05 as compared with controls.
<sup>d</sup> p < 0.01 as compared with controls.
<sup>e</sup> p < 0.05 as compared with haloperidol.

resulted in complete disappearance of 12 of the original 22 mammary tumors. When dexamethasone was administered with haloperidol, 7 of 22 tumors disappeared. In the control and haloperidol-treated groups, none of the initial tumor regressed by the end of the experiment.

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