Role of Adrenals and Estrogen in Regression of Mammary Tumors during Postpartum Lactation in the Rat

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ABSTRACT

The effects of bilateral adrenalectomy or estradiol benzoate treatment were observed on growth of 7,12-dimethylbenz(a)anthracene-induced mammary tumors during postpartum lactation. In the control and estradiol benzoate-treated postpartum lactating rats, the mammary tumors decreased approximately 40% in size by Day 5 postpartum and continued to regress to 50% of their average original diameter by Day 25 postpartum. Adrenalectomy on Day 3 postpartum prevented mammary tumor regression and resulted in renewed mammary tumor growth. By Day 10 postpartum, average mammary tumor size in the adrenalectomized rats reached prepartum diameter and continued to increase in size until Day 25. Although serum prolactin concentrations were significantly higher in the lactating rats with mammary tumors than in the nonlactating rats with mammary tumors, there were no significant differences in serum corticosterone values. Adrenalectomy resulted in a significant increase in serum prolactin levels and in a marked fall in serum corticosterone levels. It is concluded that in rats adrenocortical activity is primarily responsible for reduced mammary tumor growth during postpartum lactation.

INTRODUCTION

The paradoxical regression of mammary tumors in rats (4, 14–16) and human subjects (17) during postpartum lactation, despite high serum PRL values has been well documented, but no adequate explanation for this phenomenon has been advanced. Elevated serum PRL levels normally stimulate mammary tumor growth in rats (18, 24). During pregnancy in rats, there is increased growth of existing mammary tumors (4, 14–16) and human subjects (17) during postpartum lactation, but during lactation when serum levels of pituitary PRL also are increased by the suckling stimulus, mammary tumors regress (4, 14–16).

One possible explanation for this regression during postpartum lactation is that estrogen secretion is reduced during this period (12, 21). Since estrogen is necessary for mammary tumor growth (18), reductions in estrogen secretion during lactation could contribute to the observed tumor regression. Free glucocorticoid levels also are elevated during lactation as a result of the reduction in corticosteroid-binding globulins (6, 7) and of the suckling stimulus (23). Administration of adrenal glucocorticoids has been shown to decrease mammary tumor growth in nonlactating rats (9) and women (1, 8), whereas adrenalectomy resulted in increased tumor growth and elevated serum PRL concentrations in rats (2). In addition to their effects on PRL secretion, the glucocorticoids also may directly inhibit mammary tumor growth.

The objective of this study was to elucidate the possible role of estrogen and the adrenals on regression of mammary tumors during postpartum lactation in rats. Our results suggest that the adrenals are mainly responsible for mammary tumor regression in the lactating rats.

MATERIALS AND METHODS

Virgin female Sprague-Dawley rats (Harlan Research Animals, Indianapolis, Ind.), 55 days of age, were each given an i.v. injection of 1 ml lipid emulsion containing 5 mg of 7,12-dimethylbenz(a)anthracene. The rats were housed in plastic cages in a temperature-controlled (24°C ± 0.5°C) and light-controlled (14 hr light and 10 hr dark) room and were fed rat chow (Ralston-Purina Co., St. Louis, Mo.) and water ad libitum. When the tumors first began to appear, approximately 6 weeks after 7,12-dimethylbenz(a)anthracene administration, 4 females per cage were placed with 1 male and allowed to mate at will. The presence of sperm in the vagina, as determined by daily vaginal smears, was considered to be a positive indication of pregnancy. Impregnated females were placed in separate cages and were allowed to bear their young.

Three days after parturition, lactating rats containing one or more palpable tumors, at least 1 cm in average tumor diameter, were divided into 3 groups and treated as follows: Group 1 (intact controls) was given a daily s.c. injection of 0.1 ml corn oil vehicle; Group 2 was given a daily s.c. injection of 1 μg EB in 0.1 ml corn oil; Group 3 was bilaterally adrenalectomized and given a daily s.c. injection of 0.1 ml corn oil. All adrenalectomized rats were given 0.9% NaCl in drinking water, and the diet was supplemented with sugar cubes to help maintain normal blood sugar and electrolyte levels. A fourth group of nonpregnant, nonlactating, tumor-bearing rats served as a second control group.

Tumor measurements and body weights were observed at 2- to 4-day intervals from the day of parturition to Day 25 postpartum. Average tumor diameter for each rat was determined by using the mean of the 2 largest diameters of each spherical or oval tumor as measured with vernier calipers. In the few cases when tumors were of irregular conformations, 3 or 4 measurements of diameter were made and averaged. Blood samples were taken from all rats on the morning of Days 3 (pretreatment), 8, 14, and 20 postpartum by orbital sinus puncture under light ether anesthesia. It is assumed that the
mammary tumor growth during lactation. After 25 days of lactation, average tumor diameter was reduced by almost 50% in both the control lactating rats and the EB-treated lactating rats, as compared to initial measurements at the time of parturition. In the adrenalectomized lactating group, average tumor diameter increased nearly 40% over prepartum measurements. The growth rate of tumors in the adrenalectomized lactating rats between Days 5 and 15 postpartum was similar in intact, nonlactating control rats.

The effects of the different treatments on average tumor number are shown in Table 1. Rats in the intact lactating group showed no significant change in average tumor number during lactation. By contrast, the EB-treated lactating rats showed a significant decrease in average tumor number by the end of the 21-day treatment period, whereas a significant increase in average tumor number was observed in the adrenalectomized lactating group.

The effects of the various treatments on serum PRL levels are shown in Table 2. When compared with the nonlactating controls, serum PRL levels are elevated in all lactating groups as a result of the suckling stimulus. Serum PRL concentrations in the adrenalectomized group were higher than those in the intact, control lactating group during the first 2 weeks of lactation in these rats was measured by uniformly reducing litter size to 8 pups per mother on Day 2 postpartum and by weighing the 8 pups every 2 days. This has been a standard procedure for measuring lactation in postpartum rats (3). Statistical differences in serum PRL and corticosterone values and in tumor diameters between control and treatment groups were tested by Student's t test. A difference of $p < 0.05$ was considered to be significant.

**RESULTS**

Chart 1 shows the effect of the different treatments on mammary tumor growth during lactation. After 25 days of lactation, average tumor diameter was reduced by almost 50% in both the control lactating rats and the EB-treated lactating rats, as compared to initial measurements at the time of parturition. In the adrenalectomized lactating group, average tumor diameter increased nearly 40% over prepartum measurements. The growth rate of tumors in the adrenalectomized lactating rats between Days 5 and 15 postpartum was similar in intact, nonlactating control rats.

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**Table 1**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rats</th>
<th>Tumor no./rat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 0</td>
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<tr>
<td>Nonlactating controls</td>
<td>9</td>
<td>4.7 ± 0.6&lt;sub&gt;a&lt;/sub&gt;</td>
</tr>
<tr>
<td>Lactating controls</td>
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<td>4.4 ± 1.3</td>
</tr>
<tr>
<td>Lactating + EB</td>
<td>7</td>
<td>4.4 ± 0.6</td>
</tr>
<tr>
<td>Lactating + adrenalectomy</td>
<td>7</td>
<td>4.7 ± 0.7</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean ± S.E.
<sup>b</sup> p < 0.05 when compared with Day 0.
<sup>c</sup> p < 0.01 when compared with Day 0.

**Table 2**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rats</th>
<th>Day 3 (pretreatment)</th>
<th>Serum PRL levels (ng/ml)</th>
<th>Day 8</th>
<th>Day 14</th>
<th>Day 20</th>
</tr>
</thead>
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<td></td>
<td></td>
<td></td>
<td>Day 8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonlactating controls</td>
<td>5</td>
<td>33 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28 ± 3</td>
<td>25 ± 6</td>
<td>40 ± 12</td>
<td></td>
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<tr>
<td>Lactating controls</td>
<td>7</td>
<td>468 ± 120&lt;sup&gt;b&lt;/sup&gt;</td>
<td>619 ± 98&lt;sup&gt;b&lt;/sup&gt;</td>
<td>256 ± 57&lt;sup&gt;c&lt;/sup&gt;</td>
<td>235 ± 80&lt;sup&gt;c&lt;/sup&gt;</td>
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</tr>
<tr>
<td>Lactating + EB-treated</td>
<td>7</td>
<td>590 ± 167&lt;sup&gt;d&lt;/sup&gt;</td>
<td>350 ± 88&lt;sup&gt;d&lt;/sup&gt;</td>
<td>306 ± 125&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td></td>
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<tr>
<td>Lactating + adrenalectomy-</td>
<td>7</td>
<td>368 ± 62&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1310 ± 172&lt;sup&gt;b&lt;/sup&gt;&lt;sup&gt;c&lt;/sup&gt;</td>
<td>886 ± 150&lt;sup&gt;c&lt;/sup&gt;</td>
<td>391 ± 123&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>treated</td>
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</tr>
</tbody>
</table>

<sup>a</sup> Mean ± S.E.
<sup>b</sup> p < 0.001 when compared with nonlactating controls.
<sup>c</sup> p < 0.01 when compared with lactating controls.
<sup>d</sup> p < 0.001 when compared with lactating controls.
<sup>e</sup> p < 0.05 when compared with lactating controls.

**Figure 1**

Chart 1. Effects of adrenalectomy (Adrenx) and EB on percentage of change in average tumor diameter during lactation. Non-lact., nonlactating; Lact., lactating; Part, parturition; tx, time of treatment; Ave., average.
lactation but returned to control levels by the third week of treatment. Serum PRL levels in the EB-treated lactating rats did not differ significantly from the lactating control rats.

Bilateral adrenalectomy reduced total serum corticosterone to nearly undetectable levels. However, there were no significant differences in serum corticosterone between lactating and nonlactating controls. Both EB treatment and adrenalectomy decreased lactation as indicated by the reduced growth rate of pups when compared with pups from control lactating rats (Chart 2).

**DISCUSSION**

This study demonstrates that adrenalectomy during postpartum lactation completely prevented regression of mammary tumors in rats and resulted in tumor growth equal to that of nonlactating, intact controls. These observations suggest that the adrenals inhibit mammary tumor growth during postpartum lactation in rats. Serum PRL levels in the adrenalectomized rats were higher than those in intact lactating rats for the first 2 weeks after parturition and probably contributed to the reversal of mammary tumor regression seen in the intact, control lactating rats. In a previous study, our laboratory reported that adrenalectomy of nonlactating rats resulted in enhanced mammary tumor growth and elevated serum PRL concentrations, whereas cortisol administration inhibited mammary tumor growth and lowered serum PRL values (2).

Removal of any direct inhibitory effect by adrenal glucocorticoids on mammary tumor tissue also may have contributed to tumor growth. A preliminary study in our laboratory indicated that dexamethasone, a synthetic glucocorticoid, inhibited mammary tumor growth while depressing basal serum PRL levels. However, when serum PRL was elevated by haloperidol, dexamethasone still induced regression of mammary tumors. These observations suggest that dexamethasone, or a physiological glucocorticoid, such as corticosterone, in the rat may directly inhibit growth of mammary tumors and thus reverse any stimulatory effect exerted by elevated PRL levels. Similarly, high doses of estrogen induced regression of mammary tumors in rats and at the same time elevated serum PRL concentration. The inhibitory action of large doses of estrogen on mammary tumor growth was shown to be exerted directly on the tumor tissue, resulting in a decrease in specific PRL receptors and thereby preventing PRL from exerting its normal growth-stimulating action. Whether glucocorticoids can similarly reduce PRL receptors in mammary tumor tissue remains to be demonstrated.

Serum corticosterone levels in the intact lactating rats were similar to those of the nonlactating controls, in agreement with a previous report by Stern and Voogt (22), who compared total serum corticosterone levels in nonlactating and lactating rats. However, they also found that, after the pups had been removed from the mothers for 12 hr and then permitted an acute period of suckling by the pups, total serum corticosterone levels were significantly elevated above nonlactating control values. In the present study, blood was collected while the pups were with their mothers constantly, and any elevations in serum corticosterone as a result of acute suckling episodes may have been missed.

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Treatment with the single dose of EB given did not prevent mammary tumor regression during lactation, nor did it significantly alter serum PRL levels when compared with lactating controls. It is possible that the small dose of estrogen administered may not have been sufficient to replace the low estrogen secretion reported during postpartum lactation in rats (12). Reduced milk secretion as indicated by decreased pup weight gain in the EB and adrenalectomized rats is not believed to have been responsible for either the increased mammary tumor growth observed after adrenalectomy or the decreased mammary tumor growth observed in the intact estrogen-treated rats, which was the same as in the control lactating rats.

The present observations indicate that the adrenal glands during postpartum lactation are mainly responsible for the regression of mammary tumors observed in these rats. Whether regression of breast cancer in women during postpartum lactation is also related to adrenocortical function remains to be demonstrated, but there is evidence that glucocorticoid secretion is increased during lactation in women (13).

**ACKNOWLEDGMENTS**

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**REFERENCES**

Mammary Tumor Regression during Lactation

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