Stimulation of Carcinogen-induced Mammary Tumor Growth in Rats by Adrenalectomy

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SUMMARY

The effect of adrenalectomy on 7,12-dimethylbenz(a)anthracene-induced mammary tumor growth was studied in Sprague-Dawley female rats. Weekly measurements revealed that adrenalectomy significantly increased both mammary tumor size and number and elevated serum prolactin levels as compared to the intact controls. Daily injection of 1 mg hydrocortisone acetate into the intact 7,12-dimethylbenz(a)anthracene-tumor bearing rats did not significantly alter tumor size, number, or serum prolactin levels but, when injected into adrenalectomized rats, it prevented increased tumor growth and prolactin release. Daily injection of ovine prolactin and hydrocortisone suppressed endogenous prolactin release but significantly increased tumor size and number. Ergocornine, a prolactin-inhibiting drug, blocked adrenalectomy-induced tumor growth and partially blocked prolactin release. These results indicate that adrenalectomy in rats stimulates tumor growth by increasing prolactin release.

INTRODUCTION

PRL<sup>2</sup> and estrogen are considered to be the 2 most important hormones involved in mammary tumorigenesis in female rats (6, 7). Treatment with appropriate CNS-acting drugs (ergots, L-dopa, iproniazid) (7, 13-15) or surgical procedures (ovariectomy or hypophysectomy) (7, 11, 15) that reduce serum PRL levels results in regression of mammary tumors, whereas CNS-acting drugs (reserpine, chlorpromazine, haloperidol, etc.) that increase serum PRL levels (7, 13, 14) or placement of lesions in the median eminence (19) or grafting of extra pituitaries (18) result in enhanced mammary tumor growth in rats.

The combination of ovariectomy and adrenalectomy has been reported to induce mammary tumor remission in humans and rats (7, 11), but the effects of adrenalectomy alone on mammary tumor growth in rats have received little or no attention. After this work was completed, Clifton et al. (3) reported that adrenalectomy of irradiated multiparous female rats carrying a pituitary tumor graft showed an increased incidence of mammary tumors, and attributed this to prevention of milk production and enhancement of cellular proliferation. Ben-David et al. (2) observed that adrenalectomy of rats resulted in enhanced PRL release. The objective of this study was to determine the effects of adrenalectomy alone or in combination with hydrocortisone acetate or ergocornine (a drug that inhibits PRL release) on growth of carcinogen-induced mammary tumors and on serum PRL levels.

MATERIALS AND METHODS

Virgin female Sprague-Dawley rats were obtained from Spartan Research Animals, Haslett, Mich. They were housed in an air-conditioned, temperature- (75 ± 1°F) and light- (14 hr of light from 5 a.m. to 7 p.m.) controlled room and were fed a diet of Purina rat chow (Ralston Purina Co., St. Louis, Mo.) and water ad libitum. At 55 or 56 days of age, each rat received a single tail-vein injection of 1 ml of a lipid emulsion containing 5 mg of DMBA. Approximately 60 days later, when each rat had developed at least 1 mammary tumor 1 cm in diameter or larger, the rats were divided into 6 groups. After a pretreatment blood sample was collected, the rats were given daily s.c. injections and were treated for 3 weeks as follows: Group 1, intact controls, 0.2 ml of 0.87% NaCl solution daily; Group 2, bilateral adrenalectomy and 0.2 ml of 0.87% NaCl solution daily; Group 3, 1 mg HC per rat in 0.2 ml 0.87% NaCl solution daily; Group 4, bilateral adrenalectomy and 1 mg HC per rat in 0.87% NaCl solution daily; Group 5, 1 mg ovine PRL and 1 mg HC per rat in 0.2 ml 0.87% NaCl solution daily; Group 6, adrenalectomy and 0.2 mg EC per 100 g body weight, first dissolved in 3% alcohol and injected in 0.2 ml 0.87% NaCl solution daily. Adrenalectomized rats were given 0.87% NaCl solution in their drinking water.

Every 7 days during the 3-week treatment period, the rats were placed under light ether anesthesia, and a blood sample was collected by ocular sinus puncture. The 2 largest diameters of each tumor were measured with calipers and both size and number of tumors were recorded. At the end of the treatment period, the average tumor diameter and number of tumors/rat were calculated. Serum was separated and stored at −20° until it was radioimmunoassayed for PRL (9). PRL values are expressed in terms of NIAMDD-Rat-Prolactin-RP-1. Significance of differences in both tumor measurements and PRL levels between pre- and post-
treatment was tested by the paired t test. The differences in tumor diameter and PRL levels between any 2 groups were tested by Student's t test.

RESULTS

The effects of different treatments on mammary cancer growth are shown in Table 1. In the intact controls, there was an increase in average tumor diameter from 8.7 ± 1.7 cm (mean ± S.E.) to 12.1 ± 2.3 cm and in average tumor number from 4.8 ± 0.4 to 6.2 ± 0.9. In the adrenalectomized group, tumor diameter increased from 8.5 ± 1.4 to 18.8 ± 2.7 cm, and in average tumor number, from 4.6 ± 0.9 to 8.1 ± 0.7. In the HC group, average tumor diameter increased slightly from 8.7 ± 0.9 to 10.8 ± 1.5 cm, and average tumor number increased from 4.4 ± 0.4 to 6.1 ± 1.0. The combination of HC and adrenalectomy overrode the stimulatory effect of adrenalectomy alone. HC and PRL together increased average tumor diameter from 8.7 ± 1.1 to 6.8 ± 0.5 cm and average tumor number from 4.3 ± 0.9 to 8.1 ± 1.2. The combination of adrenalectomy with EC slightly decreased average tumor diameter from 8.7 ± 1.1 to 6.8 ± 0.5 but did not change the average tumor number.

In order to obtain a better evaluation of the effectiveness of the different treatments, percentage changes in average tumor diameter (Chart 1) and average tumor number (Chart 2) were calculated. In the control group there was an average gain of 40% in tumor diameter and 33% in average tumor number during the treatment period, whereas in the adrenalectomized group there was an average increase of 123% in tumor diameter and 100% in tumor number. Average tumor diameters in the HC-treated group increased a mere 18% and average tumor number increased 38%. The combination of adrenalectomy and HC caused no change in average tumor diameter and a slight increase (13%) in average tumor number. Average tumor diameter increased 98% and average tumor number increased 90% in the group treated with PRL and HC. The combination of adrenalectomy and EC decreased average tumor diameter by 16% and reduced average tumor number by 15%.

The effects of the different treatments on serum PRL levels are shown in Table 2. In the intact control group, serum PRL showed no significant changes throughout the 3-week period, with a beginning value of 22 ± 5 and a final value of 21 ± 3 ng/ml. Adrenalectomy increased serum PRL from 27 ± 3 to 98 ± 4 ng/ml. HC caused a slight but insignificant decrease in serum PRL from 29 ± 3 to 18 ± 3 ng/ml. The combination of adrenalectomy and HC induced a significant decrease in serum prolactin from 18 ± 5 to 10 ± 1 ng/ml. The most dramatic reduction in PRL levels was observed in the group treated with HC and ovine PRL, which decreased from a pretreatment level of 23 ± 6 to 4 ± 1 ng/ml. The combination of adrenalectomy and EC did not cause a significant change from pretreatment serum PRL levels after 1 or 3 weeks of treatment. The cause for the Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rats</th>
<th>Tumor diameter/rat (cm)</th>
<th>Tumor no./rat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wk 0</td>
<td>Wk 1</td>
<td>Wk 2</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>8.7 ± 1.7</td>
<td>10.3 ± 1.9</td>
</tr>
<tr>
<td>Adrenx</td>
<td>6</td>
<td>8.5 ± 1.4</td>
<td>12.1 ± 2.0</td>
</tr>
<tr>
<td>HC</td>
<td>7</td>
<td>8.7 ± 0.9</td>
<td>9.2 ± 1.0</td>
</tr>
<tr>
<td>Adrenx + HC</td>
<td>7</td>
<td>8.6 ± 0.9</td>
<td>7.7 ± 1.3</td>
</tr>
<tr>
<td>HC + PRL</td>
<td>7</td>
<td>8.6 ± 1.7</td>
<td>12.4 ± 0.6</td>
</tr>
<tr>
<td>Adrenx + EC</td>
<td>6</td>
<td>8.7 ± 1.1</td>
<td>7.0 ± 1.0</td>
</tr>
</tbody>
</table>

*a Adrenx, adrenalectomy.

b Mean ± S.E.

c p < 0.05 when compared with control.

d p < 0.05 when compared with Wk 0.

p < 0.001 when compared with Wk 0.
significant increase at the end of 2 weeks is unknown but may have been due to stress at the time of blood collection.

**DISCUSSION**

This work demonstrates that adrenalectomy increased DMBA-induced mammary cancer growth in rats and was associated with increased serum PRL levels. This corroborates the report of Ben-David et al. (2) that adrenalectomy in rats results in elevation in serum PRL values and appears to be in agreement with the observation of Clifton et al. (3), although the latter study was complicated by the presence of a pituitary tumor graft which secretes relatively huge amounts of PRL (5). The observation that adrenalectomy induced pseudopregnancy in rats (17) is consistent with the high prolactin levels noted in adrenalectomized rats in the present study. Since procedures that produce increased PRL secretion result in increased mammary tumor growth in rats (18, 19), it can be concluded that the enhanced mammary tumor growth observed in the present study was due to enhanced PRL release.

The rise in prolactin following adrenalectomy may result from a lack of glucocorticoids, since HC injections prevented the adrenalectomy-induced rise in prolactin. The HC dose used was approximately 5 times the recommended maintenance dose for rats (1) and, therefore, may not necessarily reflect a physiological effect of corticoids on prolactin release. Previously, Meites and Nicoll (8) observed inhibition of in vitro prolactin release from rat pituitaries incubated with cortisol in the medium, suggesting the possibility that glucocorticoids may act directly on the pituitary. Furthermore, adrenalectomy was reported to produce an increase in MAO activity in the rat brain, and this increase was prevented by HC injections (10). Since MAO is a primary degradative enzyme for catecholamines, and hypothalamic catecholamines decrease prolactin release (16), the adrenalectomy-induced increase in MAO activity could account in part for the increase in prolactin release.

Administration of 1 mg HC per rat daily had no effect on mammary tumor growth and slightly depressed serum PAL levels. However, we have recently found that injection of 2 mg HC per rat severely inhibited mammary tumor growth and reduced serum PRL values (unpublished observations). Although the 1-mg dose of HC had no effect on mammary tumor growth in intact rats, it completely prevented the stimulatory effects of adrenalectomy on tumor growth and on PRL release. Administration of PRL together with HC stimulated mammary tumor growth to about the same extent as did adrenalectomy alone, even though endogenous PRL release was suppressed. Injections of PRL previously were shown to promote mammary tumor growth in rats (6) and to decrease PRL release by the in situ pituitary through a "short-loop" feedback mechanism (7). The injected ovine PRL does not cross-react with rat PRL (9) and hence did not interfere with the assay of rat serum PRL. Although prolac-

![Chart 2. Effects of adrenalectomy, HC, adrenalectomy and HC, PRL and HC, and adrenalectomy and EC on percentage change in mammary tumor number in female rats, ADRENX, adrenalectomy.](image)

**Table 2**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rats</th>
<th>Serum PRL levels (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wk 0</td>
<td>Wk 1</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>22 ± 5†</td>
</tr>
<tr>
<td>Adrenx</td>
<td>6</td>
<td>27 ± 3</td>
</tr>
<tr>
<td>HC</td>
<td>7</td>
<td>29 ± 3</td>
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<tr>
<td>Adrenx + HC</td>
<td>7</td>
<td>18 ± 5</td>
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<tr>
<td>HC + PRL</td>
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<td>23 ± 6</td>
</tr>
<tr>
<td>Adrenx + EC</td>
<td>6</td>
<td>21 ± 6</td>
</tr>
</tbody>
</table>

*Adrenx, adrenalectomy.
†Mean ± S.E.
‡p < 0.01 as compared with control.
* p < 0.05 as compared with pre-treatment.
†p < 0.01 as compared with pre-treatment.
‡p < 0.05 as compared with control.
* p < 0.02 as compared with control.
tin levels in the adrenalectomized + EC group were generally lower than in the adrenalectomized animals, there is a suggestion that the prolactin rise induced by adrenalectomy was not entirely inhibited by EC. The decrease in tumor size and number observed in the adrenalectomy and EC group therefore cannot be attributed to a decrease in PRL levels alone. It is possible that EC also exerts a direct inhibitory effect on tumor growth, as suggested by the well-known vasoconstrictive properties of ergot drugs.

Adrenalectomy has been reported to induce remission of breast cancer in women (4, 12), in contrast to its effect in rats. However, adrenalectomy in women usually has been performed together with or after oophorectomy in order to remove the remaining source of estrogen, whereas in our rat study the ovaries were not removed and the rats continued to cycle. Estrogen is an essential hormone for mammary tumor growth in the rat (6), and the combination of ovariectomy and adrenalectomy results in mammary tumor regression in rats (11), as in women. The effect of adrenalectomy alone on PRL release in women has not yet been reported, to our knowledge.

ACKNOWLEDGMENTS

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REFERENCES

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