Estrogen Inhibition of Mammary Tumor Growth in Rats; Counteraction by Prolactin (35760)

JOSEPH MEITES,[1] ELDON CASSELL, AND JAMES CLARK

Department of Physiology, Michigan State University, East Lansing, Michigan 48823

The ovaries and anterior pituitary are essential for development and growth of spontaneous or carcinogen-induced mammary tumors in rats under most conditions (1–5). Ovariectomy inhibits, whereas administration of estrogen to ovariectomized rats permits development and growth of these tumors. In rats with developed mammary tumors, administration of large doses of estrogen results in tumor regression (6, 7). Treatment with large doses of estrogen (or androgen) also induces remission of human breast cancer in more than 20% of treated patients (8). The mechanism(s) by which large doses of estrogen inhibit mammary tumor growth has not been elucidated, and is the subject of the present investigation.

Estrogen is a potent stimulator of prolactin release in rats. Although small doses are more effective than large doses in raising blood prolactin levels, there is no evidence that even large doses of estrogen can inhibit prolactin release (9). Work in our laboratory on rats and rabbits suggests that large doses of estrogen can depress lactation by inhibiting the peripheral action of prolactin on the mammary gland [(10); Amenomori and Meites, unpublished]. Administration of prolactin largely overcame the inhibiting action of estrogen on lactation. It was of interest therefore to determine whether estrogen-induced inhibition of growth of established mammary adenocarcinomas in rats could be overcome by administering prolactin.

Materials and Methods. Fifty-five-day-old Sprague-Dawley female rats (Spartan Research Animals, Haslett, Mich.) were given a single iv injection of a lipid emulsion containing 5 mg of 7,12-dimethylbenzanthracene (DMBA).[2] This procedure previously was shown to result in relatively rapid development of mammary adenocarcinomas in Sprague-Dawley female rats (6). Beginning 4 weeks after treatment, the rats were palpated once weekly for appearance of mammary tumors. Approximately 3 months later, when most rats had mammary tumors, the tumor-bearing rats were divided into 3 similar groups and treated for 20 days as follows: (1) controls, injected once daily sc with 0.2 ml of corn oil; (ii) 20 μg of estradiol benzoate (EB) injected sc once daily in 0.2 ml of corn oil; (iii) 20 μg of EB and 1 mg of ovine prolactin[3] daily, the latter injected sc in 0.2 ml of 0.9% saline. The 20-μg dose of EB was chosen because we had previously found that this dose significantly inhibited growth of DMBA-induced mammary tumors in rats (Nagasawa and Meites, unpublished). The total number of tumors per rat and largest diameter per tumor (measured by calipers) were recorded every 5 days. The rats were maintained in a temperature (75 $\pm$ 2°F) and light-controlled (14 hr light daily) room, and given food and water *ad libitum*. Significance of differences between groups was determined by Student's *t* test.

Results. The effects of the different treatments are shown in Table I. The controls showed an average gain in number of tumors from 2.4 $\pm$ 0.4 to 3.4 $\pm$ 0.7 by the end of 20 days, and an increase in mean total tumor diameter per rat from 7.0 $\pm$ 1.2 to 10.7 $\pm$ 1.6 cm. By contrast, the rats injected with 20

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TABLE I. Effects of Estradiol Benzoate (EB) and Prolactin on Growth of DMAB-Induced Mammary Tumors.

Treatment + no. of rats	Days 0	5	10	15	20
Controls (12)					
M no. of T[h]	2.4 ± 0.4	2.8 ± 0.3	3.2 ± 0.5	2.9 ± 0.6	3.4 ± 0.7[a]
MTD (cm)	7.0 ± 1.2	8.1 ± 1.3	9.1 ± 1.3	9.0 ± 1.6	10.7 ± 1.6[c]
Change in MTD (%)		16.0	30.0	28.6	50.3
EB (10)					
M no. of T	2.5 ± 0.4	2.8 ± 0.5	2.6 ± 0.5	1.9 ± 0.3	1.7 ± 0.3[d]
MTD (cm)	13.1 ± 2.1	14.1 ± 2.5	13.7 ± 2.5	12.8 ± 2.7	12.6 ± 3.0
Change in MTD (%)		7.6	2.0	—2.3	—3.7
EB + Prolactin (10)					
M no. of T	2.9 ± 0.3	3.0 ± 0.4	3.4 ± 0.5	3.1 ± 0.5	3.2 ± 0.4
MTD	9.5 ± 1.3[f]	10.6 ± 1.3	12.5 ± 1.7	13.1 ± 1.8	14.2 ± 1.6[g]
Change in MTD (%)		16.8	31.5	37.7	49.5

[a/d] $p < .05$; [d/e] $p < .01$; [b/c] $p < .1$; [f/g] $p < .05$.
[h] T = tumors; MTD = mean tumor diameter.

μg of EB daily showed a loss in average number of tumors per rat from 2.5 ± 0.4 to 1.7 ± 0.3, and a slight but insignificant reduction in average total tumor diameter from 13.1 ± 2.1 to 12.6 ± 3.0 cm. The rats given both EB and prolactin daily showed a significant gain in mean total tumor diameter per rat from 9.5 ± 1.3 to 14.2 ± 1.6 cm and a slight but insignificant gain in average total number of tumors per rat. The percentage change in mean total tumor diameter showed a 50.3% gain in the controls, a loss of 3.7% in the EB-treated rats, and a 49.5% gain in the EB–prolactin-treated rats. The 3 groups of rats did not differ significantly in body weight by the end of the treatments.

Discussion. The present study shows that a dose of 20 μg of EB daily effectively inhibits growth of DMBA-induced mammary tumors in rats, in agreement with previous observations (Nagasawa and Meites, unpublished). Both the average number of tumors and mean total tumor diameter per rat were reduced in the EB-treated group, whereas control rats showed increases in both parameters. Daily injections of 1 mg of prolactin largely overcame the inhibition by EB and resulted in a significant increase in average total tumor diameter. Previously we found that daily injections of 20 μg of EB to Sprague-Dawley female rats with DMBA-induced mammary tumors increased serum prolactin values from 27.3 ± 2.6 to 177 ± 21.0 ng/ml (Nagasawa and Meites, unpublished). Daily injections for 5 days of 10 or 50 μg of EB into ovariectomized Sprague-Dawley rats increased serum prolactin levels to about 250 ng/ml compared to about 20 ng/ml in control ovariectomized rats (9). Thus, inhibition of mammary tumor growth by large doses of EB in rats cannot be attributed to a decrease in blood prolactin levels.

The present results suggest that a large dose of estrogen interferes with the peripheral action of prolactin on mammary tumor tissue. Administration of estrogen to lactating rats or rabbits apparently inhibited lactation by a similar mechanism, since injections of prolactin were able to partially overcome the effects of estrogen [(10); Amenomori and Meites, unpublished]. How excessive doses of estrogen interfere with the mammary tumor-promoting action of prolactin is unknown, but they may retard entry of prolactin into the mammary tumor cells or otherwise interfere with the ability of prolactin to stimulate mammary tumor growth.

Summary. Daily injection of 20 μg of estradiol benzoate for 20 days into female Sprague-Dawley rats with DMBA-induced mammary adenocarcinomas, produced a decrease in average number and mean total tumor diameter per rat. Control rats not treated with estrogen showed an increase in

average number and total tumor diameter per rat. When rats were injected daily with both estrogen and 1 mg of ovine prolactin for 20 days, there was no reduction in average number of tumors and a significant increase in average total tumor diameter per rat. These results suggest that large doses of estrogen directly inhibit stimulation by prolactin of mammary tumor growth.

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