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Effects of Hypothalamic Lesions on Incidence and Growth of Mammary Tumors in Carcinogen-Treated Rats* (32847)

JAMES A. CLEMENS[1] CLIFFORD W. WELSCH[2] AND JOSEPH MEITES
Department of Physiology, Michigan State University, East Lansing, Michigan 48823

The onset and growth of mammary adenocarcinomas in female rats given carcinogens apparently require both anterior pituitary and ovarian hormones. Thus rats ovariectomized or hypophysectomized prior to carcinogen treatment develop few or no mammary tumors (1), and ovariectomy or hypophysectomy after mammary tumor development can result in tumor regression (2). Further evidence of the importance of anterior pituitary hormones, particularly prolactin, is provided by experiments showing that single or multiple pituitary transplants can increase the incidence of mammary tumors in mice (3). Prolonged injections of prolactin into intact female mice also resulted in mammary tumors (4), as did transplantation of pituitary "mammotropic" tumors in mice (5) and rats (6).

Placement of lesions in the median eminence (ME) of the tuber cinereum results in enhanced release of prolactin and in marked reduction in release of all other anterior pituitary hormones (7). It was of interest therefore, to study the effects of ME lesions on the induction and growth of mammary tumors in intact and ovariectomized rats treated with a carcinogen.

Materials and Methods. Placement of ME lesions before DMBA. Seventy-five virgin female Sprague-Dawley rats (Holtzman Co., Madison, Wisc.), all uniform in body weight were divided randomly into four groups. At 56 days of age all animals were given a single intravenous injection of 1 ml of a lipid emulsion containing 5 mg of 7,12-dimethylbenzanthracene (DMBA).[3] Additional treatments were as follows: group 1, intact controls, no treatment; group 2, intact, bilateral lesions placed in the ME at 50 days of age; group 3, ovariectomized at 64 days of age and no further treatment; group 4, placement of bilateral lesions in the ME at 50 days of age and ovariectomized at 64 days of age. After carcinogen treatment all animals were examined once weekly for a period of 6 months for development of mammary tumors. All ME lesions were produced by passing a direct current of 2 mA for 7 sec through epoxylite coated steel electrodes prepared from insect pins. The ME was located with the aid of a

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[1] NIH predoctoral trainee (GM-1121).

[2] Special Research Fellow, National Cancer Institute, National Institutes of Health, USPHS

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TABLE I. Effects of Median Eminence Lesions Placed in Rats Before Treatment with DMBA.[a]

Group and treatment	Total no. of rats	No. and % of rats with tumors	Av no. of tumors/ tumor-bearing rat	Range and mean latency period (days)
1. Control intact	22	21 (95)	3.1 ± 0.11[b]	40–175 (78 ± 5)[b]
2. Lesioned intact[e]	20	6 (30)	1.16 ± 0.03[d]	65–177 (104 ±14)[d]
3. Control ovariectomized[f]	13	7 (54)	2.33 ± 0.42[c]	102–186 (156 ± 7)[c]
4. Lesioned ovariectomized	20	0 (0)	0	0 (0)

[a] DMBA was administered on day 56.

[b,c,d] Groups possessing different superscripts are significantly different from each other ($p = .05$). Standard error of the mean is indicated for each group.

[e] ME lesion placed on day 50.

[f] Ovariectomy performed on day 64.

Stoelting sterotaxic instrument and de Groot's atlas of the rat brain. At the time of autopsy the brains were removed, fixed in neutral buffered formalin, sectioned at 10 μ and stained with cresyl violet and luxol fast blue. The location of the ME lesions was confirmed.

Placement of ME lesions after DMBA. Fifty-seven virgin female Sprague-Dawley rats were divided randomly into four groups. At 55 days of age all animals were given a single intravenous injection of 5 mg of DMBA. Additional treatments were as follows: group 1, controls, no treatment; group 2, bilateral lesions placed in the ME 75 days after DMBA treatment; group 3, ovariectomized 75 days after DMBA treatment; group 4, ovariectomized and bilateral lesions placed in the ME 75 days after DMBA treatment. All animals were examined at 0, 10, and 25 days after ovariectomy and/or placement of lesions for palpable mammary tumors. Tumors larger than 1 cm in diameter were measured with a vernier caliper. All tumors were removed at the end of each experiment, fixed in Bouin's fluid, sectioned at 4 μ and stained with hematoxylin and eosin. They were all adenocarcinomas, similar to those described by Huggins *et al.* (2) in Sprague-Dawley rats treated with DMBA.

Results. Placement of ME lesions before DMBA (Table I). The results show that ME lesions had a definite inhibitory effect on mammary tumor induction. In the intact control rats (group 1), 95% of the rats had mammary tumors when the experiment was terminated 6 months after DMBA treatment, whereas intact rats with bilateral lesions in the ME (group 2) had only a 30% incidence of mammary tumors. The ovariectomized controls (group 3) showed a 54% tumor incidence by 6 months after DMBA treatment whereas ovariectomized rats with bilateral lesions of the ME (group 4) were completely free of mammary tumors. The average number of tumors per tumor-bearing rat was significantly lower in the lesioned groups than in either of the control groups. The number of rats with tumors and average number of tumors per rat were significantly fewer in the ovariectomized controls (group 3) than in the intact controls (group 1).

Placement of ME lesions after DMBA (Table II). Prior to placement of the ME lesions most tumors were barely palpable. The ME lesions resulted in rapid growth stimulation and in appearance of a greater number of mammary tumors. At the end of 10 days, average mean diameter of measurable tumors in the lesioned intact group (group 2) increased by 7.1 mm as compared to an increase of 3.3 mm in the control rats (group 1). Ten days after ovariectomy (group 3) the average mean diameter of measurable tumors was reduced by 6.5 mm, whereas there was an increase of 2.1 mm in the average mean diameter of tumors in the lesioned ovariectomized rats (group 4). The lesioned intact rats (group 2) showed a 120% increase in number of palpable mammary tumors 10 days after treatment, whereas the intact controls (group 1) had only a 19% increase in tumors during the same period. The stimulatory action of the ME lesions was still apparent 25 days after the lesions were placed. Ovariec-

TABLE II. Effects of Median Eminence Lesions Placed in Rats 75 Days After Treatment with DMBA.

Group and treatment[a]	Total no. of rats	Av no. of palpable tumors per rat: Initial	10 days after treatment	Change (%)	25 days after treatment	Change (%)
1. Controls, intact	13	3.2 ± 0.7[b]	3.8 ± 0.9[b]	+ 19	4.3 ± 0.8[b]	+ 33
2. Lesioned, intact	12	3.5 ± 1.2	7.7 ± 2.3	+120	10.1 ± 1.7	+189
3. Controls, ovariectomized	16	4.0 ± 0.7	2.9 ± 0.5	— 27	1.4 ± 0.5	— 56
4. Lesioned, ovariectomized	16	4.6 ± 1.1	8.3 ± 1.1	+ 80	2.8 ± 0.6	— 39

[a] DMBA was administered at 55 days of age. The rats were ovariectomized and/or lesioned approximately 75 days after DMBA treatment.

[b] SE of the mean.

tomy alone resulted in significant tumor regression 10 days after surgery, and an even greater regression 25 days after surgery (group 3). When ovariectomy was combined with ME lesions (group 4), there was a striking increase (80%) in number of palpable mammary tumors during the first 10 days after treatment. However, the stimulatory effects of the ME lesions in the ovariectomized rats did not persist, since marked tumor regression was observed by the end of 25 days.

Discussion. The results of this study indicate that when ME lesions were placed in intact and ovariectomized rats prior to DMBA treatment, mammary tumor incidence was significantly decreased. On the other hand, similar lesions placed in the ME after the appearance of small, palpable mammary tumors, rapidly stimulated tumor growth and increased the total number of tumors.

The decreased incidence of mammary tumors in rats with ME lesions placed prior to DMBA treatment may be partly due to the increased release of prolactin, stimulating mammary growth but making the mammary tissue relatively refractory to the action of DMBA. We have observed that transplantation of pituitaries into intact rats prior to DMBA treatment similarly inhibits mammary tumorigenesis and at the same time stimulates mammary growth (8). In the intact rats, increased prolactin release as a result of ME lesions may stimulate progesterone secretion by the ovaries, and the combined action of the 2 hormones could promote mammary growth (9). Ovariectomy was not performed until 14 days after ME lesions were placed and 8 days after DMBA was administered. Hence the mammary glands were in an active growth phase during a critical period before and after DMBA was given. Dao (10) showed that the critical period for the presence of the ovaries occurred within 2 weeks after DMBA treatment. In intact mice *without ME lesions* transplants of normal pituitaries for prolonged periods resulted in increased incidence of mammary tumors by providing continuous prolactin stimulation. However, DMBA was not given to these mice, and continuous hormonal stimulation alone may have evoked mammary tumorigenesis.

The ME lesions placed after the appearance of mammary tumors, rapidly increased growth and development of these tumors. In addition, many new mammary tumors arose. This can be attributed to increased prolactin stimulation of the mammary glands. In agreement with these results, transplantation of pituitary "mammosomatotropic" tumors (W5 or W15) into inbred Wistar female rats was found to induce development of mammary tumors and to markedly enhance growth of these tumors upon transplantation (6). The rapidity with which the mammary tumors grew in size and number in the ME lesioned rats was striking. This was as apparent in the ovariectomized as in the intact rats during the first 10 days after placing the ME lesions. The subsequent regression of the mammary tumors in the ovariectomized ME lesioned rats, suggests that enhanced prolactin secretion was not by itself sufficient to maintain mammary tumor

development. Apparently estrogen is also necessary for optimal growth of mammary tumors in these rats. This is in agreement with other reports that both pituitary and ovarian hormones are necessary to maintain mammary tumor growth in rats (11). Estrogen is believed to act in part by promoting pituitary prolactin secretion (12).

Summary. Intact and ovariectomized Sprague-Dawley rats with ME lesions placed prior to treatment with DMBA, showed a 30 and 0% mammary tumor incidence, respectively, in contrast to a 95 and 54% mammary tumor incidence in the respective nonlesioned control groups. Intact and ovariectomized rats lesioned 75 days after DMBA treatment showed a 120 and 80% increase, respectively, in number of palpable mammary tumors 10 days after treatment. In contrast the non-lesioned intact and ovariectomized control groups showed a 19% increase and a 27% decrease in number of palpable mammary tumors, respectively. After 25 days, stimulation of mammary tumor growth was still marked in the intact-lesioned group but regression occurred in the ovariectomized-lesioned group. These results indicate that ME lesions placed before DMBA treatment inhibit mammary tumorigenesis, presumably by increasing prolactin secretion and stimulating mammary growth, thereby leaving the mammary glands refractory to DMBA; ME lesions placed 75 days after DMBA treatment are believed to promote growth and development of mammary tumors by increasing prolactin secretion.

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