Effects of a Hypothalamic Estrogen Implant on Growth of Carcinogen-induced Mammary Tumors in Rats

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SUMMARY

Fifty-five-day-old Sprague-Dawley female rats were given an i.v. injection of 5 mg 7,12-dimethylbenz(a)anthracene, and 15 to 30 days after the appearance of palpable mammary tumors the rats were given (a) an estradiol benzoate (EB) implant in the median eminence (ME), (b) an EB implant outside the ME, (c) an EB implant in the cerebral cortex, or (d) a cholesterol implant in the ME. At 25 days after implantation, the average number of tumors per rat and percentage of increase in size and total weight of tumors in rats with an EB implant in the ME were significantly greater than in all other groups. This is attributed to the observed increase in serum prolactin levels as a result of the direct action of EB on the hypothalamus, pituitary, or both.

INTRODUCTION

Mammary tumors induced in rats by carcinogenic polycyclic hydrocarbons appear to be hormone dependent, particularly on prolactin (5, 6, 9, 10). In rats with methylcholanthrene-induced mammary tumors, hypophysectomy resulted in a marked decrease in tumor size, whereas transplantation of a functional mammotrophic pituitary tumor caused resumption of mammary tumor growth and appearance of many new tumors (5). Placement of lesions in the median eminence (4) or implantation of pituitaries in the kidney capsule (13) produced enhanced levels of serum prolactin and increased growth of DMBA-induced mammary tumors in rats. An estrogen implant in the ME of rats resulted in increased pituitary prolactin content and stimulated mammary growth (11). Implantation of estrogen in the ME of postpartum lactating rats increased lactation as judged by litter weight gains (A. Yokoyama, personal communication). It was of interest, therefore, to investigate the effects of implanting estrogen in the ME on growth of DMBA-induced mammary tumors in rats.

MATERIALS AND METHODS

Immature female Sprague-Dawley rats (Spartan Animal Research, Haslett, Mich.) were used. All animals were fed commercial Wayne Lab Blox pellets (Allied Mills, Chicago, Ill.) and tap water ad libitum and were maintained in an air-conditioned (75 ± 1°F) and artificially illuminated (14 hr light from 7:00 a.m. to 9:00 p.m. daily) room. A single injection of a lipid emulsion containing 5 mg DMBA was injected into the femoral vein of each rat at 55 days of age. The rats were examined for palpable mammary tumors every 5 days, and 15 to 30 days after mammary tumor appearance (45 to 80 days after DMBA injection) they were divided into 4 groups and treated as follows: Group Ia, EB in a glass capillary tube was implanted in the ME; Group Ib, same treatment as Group Ia, but at autopsy it was found that the glass capillary tubes were not in the ME; Group II, EB was implanted in the cerebral cortex; Group III, cholesterol was implanted in the ME. EB thoroughly mixed with cholesterol at a ratio of 1:100 or cholesterol alone was tamped into one end of the glass capillary tube (23-gauge diameter), and the tip of the glass tube was implanted in the ME with the aid of a Stoelting stereotaxic instrument. This procedure was essentially the same as described by Clemens et al. (3). The total amount of implanted EB and cholesterol or cholesterol alone was 0.7 to 1 mg.

Daily vaginal smears were made on all animals throughout the experiment, beginning 10 to 15 days before implantation. Tumor size was expressed as \( \sqrt{ab} \), where \( a \) and \( b \) were the 2 major diameters of the tumor (7). Tumor number and size were recorded every 5 days. The rats with the EB implant in the ME, all in constant diestrus, were killed by decapitation 25 days after implantation. All other rats continued to show normal estrous cycles, and were killed on the 1st diestrous day after 25 days of implantation. The tumors were removed and weighed. The location of the implant in each rat was verified.

RESULTS

Chart 1 shows that there were no differences in average number of tumors (about 3) per rat among groups on the day of implantation. However, the number of tumors in Group Ia increased linearly after EB implanta-
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Chart 1. Changes in number of DMBA-induced mammary tumors in rats after implantation of EB in the ME (Group Ia), outside the ME (Group Ib), and in the cerebral cortex (Group II) or of cholesterol in the ME (Group III). Vertical bars, S.E.

Chart 2. Changes in DMBA-induced mammary tumor size after EB or cholesterol implantation. Tumor size was expressed as \( \sqrt{ab} \), where \( a \) and \( b \) were the 2 major diameters of the tumor. Only mammary tumors which appeared before implantation were measured. Numbers of tumors measured are given in parentheses.

Chart 3. Average total weight of DMBA-induced mammary tumors per rat 25 days after EB or cholesterol implantation. Number of rats is presented in each column. Vertical bars, S.E.

DISCUSSION

Implantation of EB in the ME after mammary tumors developed in DMBA-treated rats resulted in increased numbers of tumors per rat, accelerated tumor growth rate, and greater total tumor weight than in the other 3 groups implanted with EB outside of the ME or with cholesterol in the ME. The accelerated growth of mammary tumors by an EB implant in the ME is believed to be due to increased prolactin release by the anterior pituitary. Serum prolactin concentration averaged 70 ± 11 mg/ml in the rats with an EB implant in the ME, 15 ± 4 mg/ml in rats with a cholesterol implant in the ME, 19 ± 9 mg/ml in rats with an implant outside of the ME, and 16 ± 5 mg/ml in rats with an EB implant in the cerebral cortex (10). Progesterone secretion from the functional corpora lutea of the rats with EB implanted in the ME also may have contributed to the increased mammary tumor growth. McCormick and Moon (8) suggested that progesterone acts either directly or
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synergistically with prolactin to promote growth of carcinogen-induced mammary tumors in rats.

A few days after EB implantation into the ME, the regular estrous cycles previously observed by making daily vaginal smears were disturbed, and the rats showed a continuous diestrous smear, indicative of high prolactin secretion. The other 3 groups of implanted rats continued to undergo normal cycles. When capillary tubes with the same mixture of EB and cholesterol used in Groups Ia, Ib, and II were implanted subcutaneously in 7 normal cycling rats, no alteration of estrous cycles was found. Therefore, no systemic effect on mammary tumor growth can be ascribed to the EB implants in the present experiment.

Clemens et al. (4) observed that ME lesions placed before DMBA treatment inhibited mammary tumorigenesis in rats. Similar observations were made by Welsch et al. (13), who implanted multiple pituitary homografts before DMBA treatment. Thus an increase in prolactin levels before DMBA treatment inhibits mammary tumor development. Placement of lesions in the ME (4) or implantation of pituitary homografts (13) after the appearance of palpable mammary tumors in DMBA-treated rats resulted in enhanced tumor growth. Similar acceleration of tumor growth was observed in the present study after implanting EB in the ME. Although Segaloff (12) reported that prolactin-secreting pituitary tumors inhibited growth of transplanted mammary tumors in rats, Kim and Furth (5, 6) found that such pituitary tumors accelerated growth of mammary tumors. Injections of ovine prolactin also increased growth of transplanted mammary tumors in rats (12).

The increase observed in numbers of palpable tumors after an EB implant in the ME may be the result of growth of established mammary tumor cells or transformation of preneoplastic mammary cells into tumor cells. Although the occurrence of hyperplastic alveolar nodules in rats after DMBA administration has been observed by us previously (unpublished), in agreement with the reports by Beuving et al. (1, 2), it was not determined whether the EB implants increased their numbers or promoted their transformation into tumors. It is apparent, however, that growth of already established mammary tumors was increased by an EB implant in the ME.

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