The Nature of Estrogen and Prolactin Effect on Mammary Tumorigenesis

Dilip Sinha, David Cooper, and Thomas L. Dao

Department of Breast Surgery and Endocrine Research Laboratory, Roswell Park Memorial Institute, Buffalo, New York 14203

SUMMARY

Increased release of pituitary prolactin as a result of placement of electrolytic lesions in the median eminence of the hypothalamus greatly accelerated the growth rate of carcinogen-induced mammary tumors in rats. Ovariectomy in these rats induced a rapid regression of tumors in spite of high plasma levels of prolactin. Grafting a pair of ovaries into ovariectomized rats with lesions of the median eminence did not alter the levels of plasma prolactin, but it did cause a rapid resumption of tumor growth. It is concluded that the prolactin stimulus to growth of mammary tumor is dependent on the presence of ovarian hormones.

INTRODUCTION

A unique biological characteristic of a carcinogen-induced mammary cancer is dependence on prolactin for growth. Placement of an electrolytic lesion in the tuberculum of the median eminence enhances only prolactin release from the pituitary and thereby stimulates mammary tumor growth (3, 6). Welsch et al. (15) reported an increased incidence of spontaneous mammary tumors in rats after placement of a lesion in the median eminence. Several experiments suggest that prolactin can promote the growth rate of mammary tumors even in the absence of ovarian hormones (2, 7, 14). Sterenthal et al. (13) reported that estrogen failed to stimulate mammary tumor growth in ovariectomized-adrenalectomized-hypophysectomized rats, but administration of prolactin in these triply operated rats reactivated the tumor growth. Nagasawa and Yanai (9) demonstrated that mammary cancer could be induced in ovariectomized rats if they were given estrogen or prolactin or growth hormone. It was suggested that estrogen action on the mammary gland is not a prerequisite for DMBA carcinogenesis since injection of prolactin can elicit mammary tumors in the ovariectomized rats. However, we have observed that estrogen enhances mammary tumor growth apparently without increasing the secretion of prolactin from the pituitary. Thus, when minute quantities of estrogen (diethylstilbestrol) in addition to DMBA was applied directly to the mammary gland, tumors appeared much earlier, the rate of growth was much faster, and the tumors were always larger. In these experiments, there was no evidence to demonstrate increased prolactin secretion as a result of local application of minute quantities of estrogens to the mammary gland (Ref. 12; Sinha and Dao, unpublished data). We now report in this paper that a median eminence lesion induces markedly increased tumor growth by increasing pituitary prolactin release, but the prolactin-stimulated tumor growth cannot be sustained in the absence of ovarian hormones.

MATERIALS AND METHODS

Experimental. Sprague-Dawley female rats (Holtzman Co., Madison, Wis.), 55 days old, weighing 140 to 160 g, were given a single i.v. injection of 5 mg of DMBA in a lipid emulsion. When mammary tumors were palpable (ca. 1.0 cm in diameter), electrolytic lesions were produced in the median eminence of the hypothalamus in a group of 15 rats. Three weeks after the induction of median-eminence lesions, the rats in this group were divided into 3 subgroups of 5 rats each. Ovariectomy was performed in rats in 2 of these 3 subgroups, and rats in the 3rd subgroup were not given operations. When mammary tumors regressed to barely palpable size (this usually occurred in 2 to 3 weeks), ovaries were grafted into the rats in the 1st subgroup while those in the other subgroups were not grafted.

In another experimental group of 5 rats, ovariectomy was performed when their tumors were almost 1.0 cm in diameter. Fourteen days after ovariectomy, while tumors were still regressing, median-eminence lesions were produced in all these rats. The tumor growth rate in these rats was observed.

Sham operations (median-eminence lesions) were performed in 10 rats to be used as controls. These animals given sham lesions were also divided into 2 subgroups of 5 rats each. Rats in 1 subgroup were ovariectomized 3 weeks after the sham operation and those in the other group were not operated on. For comparison, there were 2 other control groups (without sham lesion); one was intact and the other was ovariectomized.

All rats were palpated for tumors once a week and tumors were measured with a vernier caliper in 2 diameters. The arithmetic mean of these 2 diameters was designated as tumor diameter. The sum of all tumor diameters in 1 rat is designated as total tumor diameter which is the mean of all the total diameters in 1 group of rats. Blood prolactin levels were determined at regular intervals in rats in all experimental groups. At the end of the experiment all rats were killed, and tumors, endocrine organs, ovarian grafts, and brains from the lesioned rats were removed, fixed, and sectioned for histological examination.

1 The abbreviation used is: DMBA, 7,12-dimethylbenz(a)anthracene.
Placement of Lesion. The electrodes were prepared from No. 1 steel insect pins, insulated with 4 coatings of epoxylite and oven baked at 150° for 3 hr after each coating. The median eminence was located with the aid of a Stoelting Stereotaxic instrument and DeGroot’s atlas of the rat brain (4). Bilateral lesions were produced by passing a direct current of 3 ma for 7 sec through the electrode.

Prolactin Radioimmunoassay. Rats were bled from the jugular vein under light ether anesthesia. The plasma obtained was kept frozen at −20° for prolactin assay. Radioimmunoassay of prolactin was done according to the method described by Niswender et al. (10). Sheep anti-rabbit globulin was purchased from Grand Island Biological Company (Grand Island, N. Y.). Anti-rat prolactin, reference standard prolactin, and rat prolactin for iodination were kindly supplied by National Institute of Arthritis and Metabolic Diseases (rat pituitary hormone distribution program).

RESULTS

Effect of Median-Eminence Lesion and Ovariectomy on Plasma Prolactin Levels. Plasma prolactin was measured in all rats in this study. In the control rats, the plasma prolactin level ranges from 76.8 to 96.8 ng/ml plasma. Ovariectomy induces prompt reduction of plasma prolactin to 25 ng/ml. Placement of lesion in the median eminence induces a marked rise in prolactin level, reaching a peak of 240 ng/ml plasma in 6 weeks, and remained at the high level throughout the experimental period (Chart 1). Interestingly, ovariectomy fails to alter plasma level of prolactin in rats with electrolytic lesions of the median eminence. grafting a pair of ovaries into the ovariectomized rats with median eminence lesions likewise has no effect on the plasma level of prolactin. These results demonstrate that prolactin release is not affected by the presence or absence of ovarian hormones once median-eminence lesions are induced in these rats.

Relationship between Plasma Prolactin Level and Mammary Tumor Growth. When an electrolytic lesion is produced in the median eminence in rats bearing DMBA-induced mammary tumors, the tumor growth is greatly enhanced. This is parallel to the rapid rise in plasma prolactin level. However, if a median-eminence lesion is induced in the ovariectomized rats with regressing tumors it fails to stimulate the tumor growth. The tumors in these rats continue to regress despite the presence of ovarian hormones once median-eminence lesions are induced in these rats.

DISCUSSION

The relationship between prolactin and estrogen in mammary tumor growth has not been precisely defined. Understanding of this relationship may have significant clinical implication in the therapy of breast cancer in humans. Data reported in this paper conclusively demonstrate that high plasma levels of prolactin in the absence of ovarian hormones is unable to sustain or enhance the growth of mammary tumors. We observed that placement of median-eminence lesions in ovariectomized rats failed to stimulate mammary tumor growth, which continues to regress in spite of increased...
Estrogen and Prolactin in Mammary Tumorigenesis

Chart 2. Effect of median-eminence (ME) lesions, ovariectomy (Ovex), and ovarian (Ov.) grafts in mammary tumor growth. The experimental groups and the animals are the same ones used for prolactin assays in Chart 1. The curves are the measurements of tumor growth rate as expressed in mean total tumor diameter. The symbols are the same as in Chart 1.

Table 1
Uterine, pituitary, and body weights in different experimental groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Uterine horns (mg)</th>
<th>Pituitary (mg)</th>
<th>Body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>195.00 ± 6.3*</td>
<td>14.20 ± 0.9</td>
<td>254</td>
</tr>
<tr>
<td>Ovariectomy</td>
<td>48.40 ± 1.2</td>
<td>15.85 ± 1.1</td>
<td>325</td>
</tr>
<tr>
<td>Median-eminence lesions</td>
<td>201.50 ± 8.5</td>
<td>10.00 ± 0.8</td>
<td>350</td>
</tr>
<tr>
<td>Median-eminence lesions + ovary</td>
<td>54.65 ± 1.5</td>
<td>11.25 ± 0.8</td>
<td>345</td>
</tr>
<tr>
<td>Median-eminence lesions + ovary</td>
<td>145.00 ± 7.2</td>
<td>12.00 ± 1.0</td>
<td>355</td>
</tr>
</tbody>
</table>

* Mean ± S.E.

prolactin secretion. Similar observation was reported earlier by other investigators (3, 14). Clemens et al. (3) also demonstrated that if median-eminence lesions and ovariectomy were done simultaneously in rats bearing DMBA-induced mammary tumors, tumor regression occurred 15 to 20 days after these operations. They attribute this to a decreased prolactin secretion as a result of estrogen withdrawal since estrogen stimulates pituitary prolactin secretion. That this is not the case is clearly shown by the data presented here. We demonstrate that prolactin secretion does not decrease after ovariectomy in rats with median-eminence lesions.

Although the results from this study do not explain the mechanism(s) by which estrogen enhances the action of prolactin, the following explanations seem plausible. It is now well established that several estrogen-responsive tissues, including the DMBA-induced mammary tumor, contain a receptor protein binding unit that binds estrogen tightly and with high stereospecificity (5). Estrogen induces the conversion of the receptor protein binding unit from the 4 S to the 6 S form in the nuclei of calf endometrium, hence the stimulation of RNA synthesis (8). It is conceivable that estrogen acts directly on the tumor cells by an interaction with a specific macromolecular species leading to an increased macromolecular synthesis, a prerequisite for cell proliferative activity. Prolactin is not mitogenic (11) and it may act indirectly as a mitogen by rendering the cells susceptible to mitogens such as ovarian hormones. The 2nd possibility is that prolactin may stimulate the ovarian hormone synthesis, particularly the progesterone. Rat prolactin possesses luteotropic activity for the maintenance of the corpus luteum. This, however, probably is not the mechanism since examination of all surviving ovaries thus far has failed to demonstrate any active corpus luteum. The 3rd possibility is that estrogen is required for making prolactin available to the mammary tumor cells for metabolic activity. It has been demonstrated that estrogen causes vascular hyperemia and increases blood flow to the tissues (16). The decreased vascular flow to the mammary gland as a result of ovariectomy may indeed decrease the supply of prolactin to the mammary tumors. It is possible to elucidate all of these postulated mechanisms by experiments.

ACKNOWLEDGMENTS

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REFERENCES

6. McCann, S. M., and Dhariwal, A. P. S. Hypothalamic Releasing
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