Prolactin and Estrogen Dependency of Rat Mammary Cancers at Early and Late Stages of Development¹

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SUMMARY

An attempt was made to separate estrogen from prolactin dependency of 7,12-dimethylbenz(a)anthracene (DMBA)-induced rat mammary tumors at 2.5 and 5 months after DMBA injection. Ovariectomy and drug and/or hormone treatments were used to produce an estrogen or prolactin deficiency for 2 weeks, followed by a 2-week period in which the deficiency was corrected. Tumors were classified as estrogen or prolactin dependent based upon regression in the absence of the hormone and resumption of growth upon hormone replacement. At 2.5 months and 5 months after DMBA injection, about 29 and 33% of the tumors, respectively, were classified as prolactin dependent, and 35 and 45%, respectively, were classified as estrogen dependent. However, the percentage of estrogen-dependent tumors was reduced to 2.2 and 9.7%, respectively, when prolactin levels were maintained after ovariectomy. These results indicate that most DMBA-induced mammary tumors in Sprague-Dawley female rats are dependent on both estrogen and prolactin but that ovariectomy or estrogen administration do not accurately reflect estrogen dependency, since prolactin secretion also is altered by these procedures.

INTRODUCTION

Prolactin and estrogen are essential for the development and growth of DMBA²-induced rat mammary cancers (4, 9, 13, 17, 19). When circulating levels of these 2 hormones were altered by drug treatment or surgical manipulations, development and growth of mammary tumors were profoundly affected. Tumor response may vary with the stage of development and size of tumors (8), and established tumors may undergo spontaneous regression, independently of the prevailing hormonal state (24). Since the results of most tumor experiments are expressed in terms of average tumor response, the response of the individual tumors often is lost in the averaging process.

Previous attempts to determine the percentage of hormone-responsive tumors failed to separate clearly the effects of prolactin from estrogen growth. Since a decrease or increase in circulating estrogen causes a corresponding reduction or elevation in prolactin (14–16), it has been difficult to differentiate estrogen dependency from prolactin dependency. This study attempted to separate the effects of these 2 hormones on growth of individual DMBA-induced mammary cancers in rats.

MATERIALS AND METHODS

Virgin female Sprague-Dawley rats, 55 to 60 days of age, were given a single i.v. injection of a lipid emulsion containing 5 mg DMBA (Dr. Paul Schurr, The Upjohn Co., Kalamazoo, Mich.). After 2.5 months, 100 animals were randomly separated into groups of 20 rats each and were subjected to 4 weeks of treatment, divided into 2 phases of 2 weeks each. The treatment schedules are shown in Table 1.

Group A received injections of 0.85% NaCl solution throughout both phases of treatment and served as a control group with normal prolactin and estrogen levels. Group B was ovariectomized for the 1st phase of treatment to remove the primary source of estrogen, and 3.75 μg EB were given daily during the 2nd phase to correct the estrogen deficiency. This dose was that recommended as a replacement dose in ovariectomized rats (1). Group C was given injections of 0.5 mg EC (Sandoz A.G., Basel, Switzerland) to reduce prolactin levels during the 1st phase of treatment, followed by ovariectomy and injections of 120 μg HAL [4'-fluoro-4-(4-hydroxy-4-p-chlorophenylpiperidine)butyrophenone] (McNeil Laboratories, Inc., Fort Washington, Pa.) to reduce estrogen and raise prolactin during the 2nd phase. Ergot drugs have been shown to reduce prolactin release (23), and HAL has been shown to raise serum prolactin levels in female Sprague-Dawley rats (6). Animals in Group D were 1st ovariectomized and given injections of 0.5 mg EC to reduce both estrogen and prolactin levels and were then given 3.75 μg EB and 120 μg HAL daily to increase the estrogen and prolactin levels during the 2nd phase of treatment. Group E was ovariectomized and given 120 μg HAL during the 1st phase of treatment to reduce estrogen while maintaining high prolactin levels, whereas the 2nd treatment of 3.75 μg EB and 0.5 mg EC was given to raise estrogen and reduce prolactin levels.

All injections were given s.c. in a volume of 0.2 ml daily between 10:00 and 11:00 a.m. EB and HAL were suspended in corn oil. EC was dissolved in 70% ethanol and diluted with 0.85% NaCl to a final concentration of 14% ethanol.

Pretreatment measures were made in individual rats of tumor size, number of tumors, and body weight. During the

¹ Supported in part by NIH Research Grants CA 10771, from the National Cancer Institute, and AM 04784, from the National Institute for Arthritis and Metabolic Diseases.

² The abbreviations used are: DMBA, 7,12-dimethylbenz(a)anthracene; EB, estradiol benzoate; EC, ergocornine methanesulfonate; HAL, haloperidol.

Received July 16, 1975; accepted October 17, 1975.
treatment, each tumor was measured weekly with calipers for length, width, and depth to the nearest mm in each dimension. The sum of the length, width, and depth of each tumor at the beginning of treatment was compared with the sum of the 3 diameters at the end of treatment. Each tumor was classified as growing, regressing, or stable at the end of both the 1st and 2nd treatments. A tumor that had increased by 3 mm or more in the sum of its measurements was classified as growing, while tumors that had decreased by 3 mm or more were classified as regressing. A tumor that had changed by less than 3 mm in the sum of its 3 diameters was considered stable. The 2nd phase of treatment was started immediately after the 1st 2 weeks of treatment in order to determine the effects of changing estrogen and prolactin levels in opposite directions from the 1st treatment phase. Thus the same tumor could be subjected to both hormone deprivation and replacement at approximately the same stage of development. The 2nd experiment with 100 rats began 5 months after DMBA injection and followed the same treatment schedule as in the rats started at 2.5 months after DMBA injection.

RESULTS

The results in Table 2 show that, at 2.5 months after DMBA injection, 76% of the tumors in the intact controls (Group A) showed growth during the 1st 2 weeks and 57.4% showed growth during the 2nd 2 weeks. Also, a greater percentage of the tumors regressed during the 2nd 2 weeks. In the ovariectomized rats (Group B), 89.2% of the tumors showed regression by the end of 2 weeks. When replacement with EB was given during the 2nd 2 weeks, the regression rate was reduced to 27.3% , and 41.8% of the tumors grew. Treatment with the ergot drug (Group C) resulted in regression in 64.3% of the tumors, and only 14.3% of the tumors grew. When this was followed by ovariectomy and HAL, regression of tumors was reduced to 32.1% and tumor growth increased to 41.5%. When ovariectomy and EC were combined (Group D), 96.2% of the tumors showed regression and no tumors showed growth. Reversal of this treatment with HAL and EB resulted in growth of 61.0% of the tumors. Ovariectomy and HAL administration (Group E) produced regression in 58.1% of the tumors, and only 37.2% of the tumors grew. When these rats were treated with EC and EB during the 2nd 2 weeks, 79.1% of the tumors regressed and only 11.6% of the tumors grew.

The tumor responses at 5 months after DMBA treatment are shown in Table 3 and are similar in many respects to the responses at 2.5 months after carcinogen treatment. In the intact controls (Group A), 22.0% of the tumors regressed during the 1st 2 weeks and 36.4% of the tumors regressed by the 2nd 2 weeks, indicating a higher spontaneous regression rate when compared with the controls 2.5 months after DMBA treatment. Also, fewer tumors showed growth at 5 months after DMBA administration. Ovariectomy (Group B) resulted in 75.6% of the tumors regressing and 12.2% growing at 5 months after DMBA treatment. Also, fewer tumors showed growth at 5 months after DMBA administration. Ovariectomy (Group B) resulted in 75.6% of the tumors regressing and 12.2% growing at 5 months after DMBA treatment. Also, fewer tumors showed growth at 5 months after DMBA administration. Ovariectomy (Group B) resulted in 75.6% of the tumors regressing and 12.2% growing at 5 months after DMBA treatment. Also, fewer tumors showed growth at 5 months after DMBA administration. Ovariectomy (Group B) resulted in 75.6% of the tumors regressing and 12.2% growing at 5 months after DMBA treatment.
the tumors regressing and only 4.3% growing. Raising the hormone levels during the 2nd treatment phase caused only 13.4% of the tumors to regress and 59.7% to grow. Ovariectomy and HAL treatment (Group E) produced regression in 34.4% of the tumors, and 49.4% grew. EB and EC given during the 2nd 2-week period caused 66.7% of the tumors to regress and 20.3% to grow.

Table 4 presents the percentage of tumors determined to be estrogen and prolactin dependent at each age. The response of each tumor to hormone changes at the end of both the 1st and 2nd stages of treatment was considered in order for a tumor to be classified as estrogen or prolactin dependent. At 2.5 months after DMBA administration, prolactin dependency was determined from the response of tumors in 2 of the treatment groups, C and E. In Group C a tumor must have both regressed in the 1st treatment phase, when prolactin was reduced by EC injection, and grown in the 2nd phase, when prolactin was raised by HAL injections.

In this group, 29.8% (16 of 56 tumors) were classified as prolactin dependent. In Group E, 28.9% (13 of 45 tumors) were judged to be prolactin dependent, based upon tumor growth in response to HAL injections of ovariectomy rats and regression when prolactin levels were depressed by EC injection during EB replacement. Estrogen dependency determinations also could be made in 2 treatment groups, B and E. At 2.5 months after DMBA injection. 35.4% (23 of 65 tumors) were considered to be estrogen dependent in Group B, because they both regressed in response to ovariectomy and grew when estrogen replacement was given. In Group E, only 2.2% showed estrogen dependency, since the tumors regressed in response to ovariectomy, when prolactin levels were maintained by HAL injections, and grew during the 2nd phase, when EB and EC were given to replace estrogen while lowering prolactin.

Tumors were classified as independent of estrogen or prolactin if they spontaneously regressed in the presence of

<table>
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<th>Table 3</th>
<th>Mammary tumor response to treatments 5 months after DMBA injection</th>
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<td>Group</td>
<td>Treatment</td>
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<tr>
<td>A</td>
<td>1. 0.85% NaCl</td>
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<td>2. 0.85% NaCl</td>
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<td>B</td>
<td>1. OVX</td>
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<td>2. OVX + EB</td>
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<td>C</td>
<td>1. EC</td>
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<td></td>
<td>2. OVX + HAL</td>
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<td>D</td>
<td>1. OVX + EC</td>
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<td></td>
<td>2. OVX + HAL + EB</td>
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<tr>
<td>E</td>
<td>1. OVX + HAL</td>
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* 1., 1st 2 weeks of treatment; 2., 2nd 2 weeks of treatment.

*Numbers in parentheses, percentages.

* OVX, ovariectomized.

<table>
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<tr>
<th>Table 4</th>
<th>Classification of tumors as prolactin or estrogen dependent 2½ and 5 months post-DMBA</th>
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<td>Group</td>
<td>Treatment</td>
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</table>
| A       | 1. 0.85% NaCl solution | 2.5 mos., 0% (0/79)
2. 0.85% NaCl solution | 5 mos., 16.4% (10/61)
| B       | 1. OVX | 2.5 mos., 35.4% (23/65)
2. OVX + EB | 5 mos., 42.9% (21/49)
| C       | 1. EC | 2.5 mos., 29.8% (16/56)
2. OVX + HAL | 5 mos., 32.8% (20/61)
| D       | 1. OVX + EC | 2.5 mos., 28.9% (13/45)
2. OVX + HAL + EB | 5 mos., 34.4% (21/62)
| E       | 1. OVX + HAL | 2.5 mos., 28.9% (13/45)
2. OVX + EC + EB | 5 mos., 9.7% (6/62)

* 1., 1st 2 weeks of treatment; 2., 2nd 2 weeks of treatment.

* Indicated by spontaneous regression in the presence of normal levels of estrogen and prolactin (0.5% NaCl controls). Numbers in parentheses, number of tumors in this classification/total tumor number.

* A tumor must exhibit the indicated response in both phases of treatment to be included in the classification.

* OVX, ovariectomized.
normal levels of estrogen and prolactin, continuously, throughout both 2-week phases of treatment. No tumors in the control group (Group A) regressed during both phases of treatment, although there were tumors that regressed during Phase 1 or 2 only but were stable or grew during the other treatment phase.

The same criteria for hormone dependency were used for tumor classification 5 months after DMBA injection. Again the response of the same tumor was considered in both phases of treatment. Prolactin dependency was found in 32.8% (20 of 61 tumors) in Group C, based upon regression and then growth in response to the 2 treatments. In Group E, prolactin dependency was found in 34.4% (21 of 62 tumors). Estrogen dependency was found in 42.9% (21 of 49 tumors) in Group B. These tumors first regressed and then grew in response to the treatments. In Group E, 9.7% (6 of 62 tumors) showed estrogen dependency by regression in response to Phase 1 of treatment and growth in response to Phase 2.

At 5 months, independent tumors, i.e., those that spontaneously regressed throughout both phases of the 4 weeks of treatment in the presence of normal levels of both hormones, made up 16.4% (10 of 61 tumors) in the controls (Group A).

**DISCUSSION**

Classification of mammary tumors based on their hormone dependency is a complex task because of the difficulty in selecting the criteria for establishing hormone dependency. In this experiment, the parameter used was that the tumor must grow in the presence of the hormone as well as regress in its absence. It is probable that such a test excludes some tumors that possess some degree of response to estrogen and prolactin, but this study did not attempt to measure degree of response. Clear growth or regression in both the presence and absence of the hormone was required to distinguish the tumors that were responding to the hormonal changes from those that grew or regressed spontaneously. It also must be emphasized that the replacement treatments with EB and HAL may not have achieved normal levels of estrogen and prolactin in these animals, and neither of these hormones was measured in these rats. The amount of estrogen given is considered to be the replacement dose for the ovariectomized rat (1), but the dose of HAL used may have raised prolactin above normal levels. Thus the reactions of the tumors to estrogen or prolactin replacement may represent responses to somewhat elevated levels of these hormones.

The percentage of regressing tumors in response to hormone changes is probably more accurately reflected when the percentage of spontaneously regressing tumors in the control rats is subtracted from the percentage of regressing tumors in each treatment group. This reduces the number of regressing tumors that can be attributed to treatment to a greater extent in the older than in the younger rats, since the older rats had a higher spontaneous regression rate. Such an adjustment makes the greater hormone responsiveness of the younger tumors even more impressive than the results with each treatment indicate.

Spontaneous mammary tumor regressions were reported in 13% of 23 untreated rats in a previous study (2). Although the age of the tumors at the time of observation was not given, the percentage falls within the range of 0% at 2.5 months and 16% at 5 months, determined in this study. Young and Cowan (24) remarked on the large number of DMB-induced rat mammary tumors that regressed spontaneously or reached a plateau in growth. They found that 27% (49 of 181 tumors) were regressing, 52% (95 of 181 tumors) were stable, and 21% (37 of 181 tumors) were growing when inspected at 26 weeks post-D Baba. These figures compare with the 36% regressing, 27% stable, and 36% growing observed at 23 to 25 weeks post-D MBA in the control rats in the present study.

Prolactin dependency has been tested in rats bearing DMB-induced mammary tumors by administration of prolactin antiserum for 36 days (2). Under these conditions, 50% (10 of 20 tumors) regressed and 35% (7 of 20 tumors) grew versus 13% (3 of 23 tumors) that regressed and 57% (13 of 23 tumors) that grew in controls not given prolactin antiserum. Thus, adjusting for controls, the percentage of prolactin-dependent tumors appears to be about 37%, which is only slightly higher than the 29% and 34% prolactin dependency determined in this study. The authors failed to state the elapsed time after D MBA administration, which may be a factor contributing to the small differences in prolactin dependency in the 2 studies. The consistency of the prolactin dependency determination in the various treatment groups argues well for the methods chosen to regulate prolactin deficiency and replacement.

Previous experiments to determine estrogen dependency in tumors have generally relied upon regression in response to ovariectomy. Teller et al. (20) found that 81% of ovariectomized D MBA-induced tumor-bearing rats experienced a decrease of 25% in the sum of all the tumor diameters per rat. These results agree remarkably well with the response to ovariectomy in the 2 age groups represented in the present study (75% and 89% regressing), which is based upon individual tumor response. Also, in a study of mammary tumors regressing after ovariectomy, Young et al. (25) found that 81% (45 of 53 rats) experienced tumor regression.

Rat mammary tumor response to ovariectomy was studied at 4, 5, and 6 months after DMBA injection by Griswald and Green (8). They found a greater and longer lasting decrease in tumor size after ovariectomy at 4 months post-D MBA than at later periods. This agrees with data in this experiment showing that 89% of the tumors regressed in response to ovariectomy in the younger group, while 75% regressed at 5 months post-D MBA.

In addition to its effects on estrogen and prolactin, ovariectomy also results in the removal of the primary source of progesterone. Progesterone has been observed to stimulate mammary tumor epithelial cell DNA synthesis in vitro (5) and mammary tumor growth in ovariectomized rats (9). Administration of progesterone alone or in combination with EB at the time of DMBA injection was able to delay the appearance and reduce the number of induced mammary carcinomas in rats (11). In view of these effects of progesterone on mammary tumor growth, the reduction of progesterone as well as estrogen and prolactin must be considered in evalu-
Mammary Tumor Response to Estrogen and Prolactin

ating the effects of ovariectomy on mammary tumor growth.

While the percentage of regressing tumors under the estrogen-reducing treatments agrees with the previous studies, the percentage that regressed when prolactin levels were maintained at a high level was greatly reduced. In the present study, estrogen dependency estimates were reduced by more than three-fourths when prolactin levels were manipulated in the opposite direction from estrogen. This confirms the need to adjust estrogen dependency figures for the effects of prolactin and tends to reduce the importance of estrogen relative to prolactin in controlling growth of DMBA-induced mammary tumors in Sprague-Dawley rats. Prolactin alone has been shown to maintain mammary tumor growth in ovariectomized-adrenalecto-

ized-hypophysectomized rats for periods up to 18 days (17). Growth of DMBA-induced mammary tumors can be maintained for at least 10 days in ovariectomized rats by placement of median eminence lesions that increased serum prolactin levels (3). These results suggested an important role for prolactin in mammary tumor regression following ovariectomy, as has been confirmed in the present study. Whether cancer regression following ovariectomy is primarily a result of prolactin reduction, a more complex interaction involving synergism of prolactin and estrogen, or an estrogen-mediated phenomenon distinct from the prolactin-stimulated growth of tumors in ovariectomized rats has not been adequately determined. However, the present observations suggest that prolactin is of greater importance than estrogen in maintaining growth of DMBA-induced mammary tumors.

A recent study of the interactions of prolactin and estrogen on mammary tumor growth concluded that estrogen may play a primary role as a result of estrogen reduction, while prolactin levels appear to have little influence on the tumor response to estrogen. While estrogen levels appear to have little influence on the tumor response to prolactin. These studies support previous investigations who minimized the influence of estrogen relative to prolactin on mammary tumor growth (17). In view of recent developments in hormone receptor research, the determination of hormonal influence on mammary cancers may be even more complicated than the present manipulations of circulating hormone levels indicate.

REFERENCES

1. Barnes, C. D., and Eitherington, L. G. Drug Dosage in Laboratory Ani-


Mammary Carcinoma in Response to Anthrohormone Treatment. Cancer


4. Dao, T. L. Role of Ovarian Hormones in Initiating the Induction of

Mammary Cancer in Rats by Polynuclear Hydrocarbons. Cancer Res.,


5. Dao, T. L., and Sinha, D. Oestrogen and Prolactin in Mammary Carci-
genesis: In Vivo and In Vitro Studies. In: A. R. Boyns and K. Griffiths


6. Dickerman, S., Clark, J., Dickerman, E., and Meites, J. Effects of Haloper-

idol on Serum and Pituitary Prolactin and on Hypothalamic PIF in Rats.


7. Gelato, M., Marshall, S., Boudreau, M., Bruni, J., Campbell, G. A., and

Meites, J. Effects of Thyroid and Ovaries on Prolactin Binding Activity in


10. King, R. B. J., Cowan, D. M., and Inman, D. R. The Uptake of (6,7-

3H)Oestradiol by Dimethylbenzanthracene-induced Rat Mammary Tu-


11. Kledzik, G. S., Bradley, C. J., and Meites, J. Reduction of Carcinogen-


12. Leung, B. S., Sasaki, G. H., and Leung, J. S. Estrogen-Prolactin Dependen-
cy in 7,12-Dimethylbenz(a)anthracene-induced Tumors. Cancer Res.,


13. Meites, J. Relation of Prolactin and Estrogen to Mammary Tumorogene-


17. Talwalker, P. K., Meites, J., and Mizuno, H. Mammary Tumor Induction by Estrogen or Anterior Pituitary Hormones in Ovariectomized Rats


176: 531—534, 1964.


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