Role of Estrogen and Prolactin in Stimulation of Carcinogen-induced Mammary Tumor Development by a High-Fat Diet

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ABSTRACT

The role of estrogen and prolactin in high-fat (HF) dietary stimulation of carcinogen-induced mammary tumors was examined in female Sprague-Dawley rats. At 55 days of age, the rats were given injections i.v. of 5 mg of dimethylbenz[a]anthracene and, 5 days later, rats were sham- or bilaterally ovariectomized. Ten days after dimethylbenz[a]anthracene administration, the rats were placed on either a 20.0% HF diet or a 4.5% control fat diet and were then subjected to various drug and endocrine treatments to maintain uniform levels of circulating estrogen and prolactin. Sham-operated intact rats and bilaterally ovariectomized rats were given daily injections of haloperidol to increase prolactin secretion; bromocryptine to decrease prolactin secretion, and/or estradiol benzoate (EB).

The intact rats fed the HF diet showed significant stimulation of all parameters of mammary tumor development when compared to similarly treated rats fed the CF diet. In ovariectomized rats fed either the HF or CF diet, there was nearly complete inhibition of mammary tumor development. When the HF diet was given to ovariectomized rats treated daily with either haloperidol or EB, EB and bromocryptine, some parameters of mammary tumor development were enhanced by the HF diet. However, in all cases, mammary tumorigenesis was reduced when compared to sham-operated control rats. Ovariectomized rats fed the HF diet and given both EB and haloperidol exhibited significantly greater tumor number per rat, increased average tumor size, and reduced tumor latency period when compared to similarly treated rats fed the CF diet. However, these parameters of mammary tumorigenesis were still reduced when compared to those of sham-control rats fed the HF diet.

These results indicate that a HF diet requires adequate circulating levels of estrogen and prolactin to maximally promote increased mammary tumorigenesis in dimethylbenz[a]anthracene-treated rats. Moreover, the enhancing effects of a HF diet on mammary tumorigenesis can be achieved in the presence of similar circulating levels of estrogen and/or prolactin, whether decreased or increased. These results suggest, therefore, that mechanisms independent of altered secretion of estrogens and/or prolactin are involved in promotion of mammary tumorigenesis by high levels of dietary fat.

INTRODUCTION

A role for dietary factors has been reported in the etiology of human breast cancer (7, 53). Epidemiological evidence has established strong positive correlations between the per capita consumption of dietary fat and the incidence of breast cancer among women (5, 7, 22, 37). Such epidemiological evidence appears to receive support from the many reports that HF diets can stimulate mammary tumorigenesis in rodent mammary tumor systems, including spontaneous, transplanted, and carcinogen-induced tumors (4, 11, 20, 21, 25, 28, 29, 46, 49). In general, animals fed a HF diet showed a higher incidence of mammary tumors, a greater number of tumors per animal, and a shorter latency period for tumor appearance than did animals fed a low-fat diet.

The mechanism(s) involved in the stimulatory effects of HF diets on mammary tumorigenesis in murine species remain to be clarified. Since most murine mammary tumor models are highly hormone dependent, particularly on estrogen and prolactin (39, 51), several reports on the mechanisms by which HF diets stimulate tumorigenesis have focused on possible mediation via PRL and/or estrogen. Chan and Cohen (10) observed that administration of CB-154 to reduce PRL secretion could abolish the stimulatory effect of HF diets on mammary tumorigenesis in rats. However, the administration of an antiestrogenic drug, nafoxidine, had a minimal effect on the stimulation of mammary tumorigenesis by a HF diet. These investigators also reported that consumption of HF diets elevated serum PRL above normal levels on the afternoons of proestrus and estrus in the cycling female rat, and they concluded that increased PRL secretion was responsible for the action of HF diets on mammary tumor development (12, 13). Reports by others, including our laboratory, have failed to show a significant influence of HF diets on blood PRL levels at any time during the estrous cycle (2, 6, 8, 27, 47). Furthermore, Ip et al. (32) reported that the differential effect of HF and low-fat diets on DMBA-induced mammary tumorigenesis could not be abolished by hypothalamic median eminence lesions, even though the circulating PRL levels were elevated similarly in both HF- and low-fat-treated animals.

The objective of the present study was to determine whether consumption of a HF diet could promote carcinogen-induced mammary tumorigenesis when circulating levels of estrogen and PRL were kept uniform in ovariectomized rats by administering a constant dose of estrogen and/or haloperidol or CB-154 to maintain a constant level of PRL in the blood. Ovariectomy is known to result in almost complete elimination of circulating estrogen and a reduction of PRL secretion (40), whereas estro-
gen administration to ovariectomized rats can restore blood estrogen levels and increase PRL secretion by the pituitary (40). Haloperidol is known to be a potent inhibitor of PRL secretion (18), whereas CB-154 inhibits PRL secretion (42). Attempts were also made in this study to determine the relative importance of estrogen and PRL in the HF stimulation of carcinogen-induced mammary tumorgenesis.

MATERIALS AND METHODS

Mammary Tumor Induction. Virgin female Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA), 55 days of age, were given injections i.v. of 1 ml of lipid gels containing 5 mg of DMBA (kindly provided by Dr. Paul Schurr, The Upjohn Co., Kalamazoo, MI). It has been the experience of this laboratory that, using this method of mammary tumor induction, over 90% of the mammary tumors that develop 4 to 5 months after DMBA administration are classified histologically as adenocarcinomas. All rats were housed in metal suspension cages in a temperature- and light-controlled room (24 ± 0.5°C (S.E.); 14 hr of light and 10 hr of dark). Most tumors became palpable 1 to 3 months after DMBA injection. All animals were given either laboratory rat chow (Purina) in pelleted form or the semisynthetic variable fat diet (Table 1) ad libitum. Tap water was also provided ad libitum.

Dietary Treatments. Beginning 10 days after DMBA administration and continuing for the duration of the experiment (16 to 23 weeks), rats were fed either the 20% HF or the 4.5% CF semisynthetic diet (Table 1). Diets were prepared at least once a week and stored at 4°C until dispensed. All rats were fed every 2 days or more often.

Endocrine and Drug Treatments. Five days after DMBA administration, the rats were either bilaterally or sham-ovariectomized. Beginning 5 days later, estrogen and PRL were maintained at constant levels in the ovariectomized rats as follows: (a) reduced levels of both estrogen and PRL were maintained in ovariectomized rats by treatment with vehicle alone; (b) physiological levels of estrogen were maintained in ovariectomized rats by daily s.c. injection of EB at a dose of 2 μg/rat; (c) physiological concentrations of estrogen and reduced levels of PRL were maintained in ovariectomized rats by daily s.c. injections of 2.0 μg of EB per rat and CB-154 (0.2 mg/kg; Sandoz, Basel, Switzerland); (d) elevated serum PRL levels and reduced serum estrogen levels were maintained in ovariectomized rats by daily s.c. injections of haloperidol (0.5 mg/kg; McNeill Laboratories, Spring House, PA); and (e) elevated serum PRL levels and physiological levels of estrogen were maintained in ovariectomized rats by daily s.c. injections of both haloperidol (0.5 mg/kg) and EB (2 μg/rat). Sham-operated rats exhibiting regular estrous cycles were used as controls.

EB and bromocryptine were suspended in 0.85% NaCl solution containing 0.3% ethanol. Haloperidol was dissolved in 0.3% tartaric acid. All drugs were administered by s.c. injection in the back of the animals in a volume of 0.1 ml.

Assessment of Mammary Tumor Development. Developing mammary tumors were palpated and measured at weekly intervals, beginning 1 month after DMBA administration. Palpable mammary tumors were measured with vernier calipers, and the 2 largest perpendicular diameters were recorded and averaged. Weekly tumor measurements were totaled for each rat and expressed as the sum of the average tumor diameter per rat for each treatment group. In addition, mammary tumor development was assessed for mean latency period, i.e., the period in weeks between DMBA administration and initial appearance of tumors. Also recorded was percentage of tumor incidence, i.e., the number of rats with tumors per total number of rats at risk, and the average number of tumors per rat at the termination of the experiment. Body weights were recorded at weekly intervals beginning 1 week after DMBA administration.

Blood Collection for RIA of PRL and Estradiol. Blood was collected 3, 9, and 16 weeks after DMBA injection by orbital sinus puncture under light ether anesthesia. Between 10 and 19 blood samples were collected in each treatment group at each sampling period. All blood samples were taken between 10 and 12 a.m., 90 min after daily drug or hormone injection. Serum from collected blood was separated by centrifugation and stored at −20°C until assayed for PRL and estradiol by standard RIA (30, 43). Antiestradiol-6-bovine serum albumin (GDN 244) was provided by Dr. G. D. Niswender.

Statistically significant increases in mammary tumor size and number and reductions in mammary tumor latency period between similarly treated rats fed either the HF or CF diet were determined by a 1-tailed Student’s t test. Significant differences in body weight and serum PRL and estradiol levels between similarly treated rats fed either the CF or HF diet were determined by a 2-tailed Student’s t test. Differences in mammary tumor incidence were evaluated by χ² test. Differences of p < 0.05 were considered significant.

RESULTS

The effects of a HF diet on DMBA-induced mammary tumor development and as influenced by normal circulating levels of estrogen and PRL in intact rats are shown in Chart 1 (top) and...
Table 2

Effects of HF and CF diets on mammary tumor development in rats given different endocrine treatments

<table>
<thead>
<tr>
<th>Group and treatment</th>
<th>Diet</th>
<th>No. of rats</th>
<th>Av. no. of tumors/rat</th>
<th>% of tumor incidence</th>
<th>Total no. of tumors</th>
<th>Mean tumor latency period (wk)</th>
<th>Mean body wt (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Sham-operated controls</td>
<td>CF</td>
<td>17</td>
<td>5.4 ± 0.9f</td>
<td>88.2</td>
<td>92</td>
<td>9.8 ± 0.3f</td>
<td>273.8 ± 5.4</td>
</tr>
<tr>
<td></td>
<td>HF</td>
<td>18</td>
<td>9.3 ± 1.3f</td>
<td>100</td>
<td>173</td>
<td>8.8 ± 0.2f</td>
<td>270.0 ± 6.6</td>
</tr>
<tr>
<td>2. Ovariectomized controls</td>
<td>CF</td>
<td>15</td>
<td>0.1 ± 0.1</td>
<td>6.7</td>
<td>1</td>
<td>10.0 ± 0.0</td>
<td>307.5 ± 7.2</td>
</tr>
<tr>
<td></td>
<td>HF</td>
<td>17</td>
<td>0.2 ± 0.1</td>
<td>11.2</td>
<td>3</td>
<td>12.2 ± 1.2</td>
<td>313.8 ± 7.7</td>
</tr>
<tr>
<td>3. Ovariectomy + EB</td>
<td>CF</td>
<td>17</td>
<td>5.4 ± 1.0</td>
<td>70.6</td>
<td>91</td>
<td>13.0 ± 0.3</td>
<td>297.2 ± 5.5</td>
</tr>
<tr>
<td></td>
<td>HF</td>
<td>14</td>
<td>5.6 ± 1.1</td>
<td>92.9</td>
<td>76</td>
<td>12.1 ± 0.4f</td>
<td>296.9 ± 5.7</td>
</tr>
<tr>
<td>4. Ovariectomy + EB + CB-154</td>
<td>CF</td>
<td>19</td>
<td>1.8 ± 0.4</td>
<td>68.4</td>
<td>35</td>
<td>15.9 ± 0.5</td>
<td>288.3 ± 8.8</td>
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<tr>
<td></td>
<td>HF</td>
<td>15</td>
<td>4.0 ± 1.0f</td>
<td>66.7</td>
<td>60</td>
<td>16.0 ± 0.5</td>
<td>303.8 ± 6.8</td>
</tr>
<tr>
<td>5. Ovariectomy + haloperidol</td>
<td>CF</td>
<td>19</td>
<td>0.3 ± 0.2</td>
<td>21.0</td>
<td>7</td>
<td>10.3 ± 2.3</td>
<td>272.6 ± 5.8</td>
</tr>
<tr>
<td></td>
<td>HF</td>
<td>17</td>
<td>1.0 ± 0.6</td>
<td>52.9</td>
<td>18</td>
<td>17.0 ± 1.0</td>
<td>279.4 ± 6.0</td>
</tr>
<tr>
<td>6. Ovariectomy + EB + haloperidol</td>
<td>CF</td>
<td>16</td>
<td>3.8 ± 0.9</td>
<td>75.0</td>
<td>64</td>
<td>14.8 ± 0.4</td>
<td>258.3 ± 6.6</td>
</tr>
<tr>
<td></td>
<td>HF</td>
<td>18</td>
<td>6.2 ± 0.7f</td>
<td>93.8</td>
<td>98</td>
<td>12.4 ± 0.4f</td>
<td>255.4 ± 6.4</td>
</tr>
</tbody>
</table>

a At 14 weeks after DMBA treatment.

b Sacrificed at 16 weeks after DMBA administration.

c Statistical comparison inappropriate due to small number of tumors.

Note: Mean ± S.E.

Table 2 (Group 1). It can be seen that mammary tumor development was enhanced in sham-operated rats fed the HF diet when compared with similarly treated rats fed the CF diet. Sham-operated rats fed the HF diet showed an increase in average tumor number and percentage of tumor incidence and a decrease in average latency period when compared with sham-operated rats fed the CF diet (Table 2, Group 1). Ovariectomy resulted in nearly complete suppression of mammary tumor development in rats fed either the HF or CF diet (Chart 1, bottom). Average tumor number and percentage of tumor incidence were reduced similarly in ovariectomized rats fed either the HF or the CF diet (Table 2, Group 2).

The effects of replacement with estrogen after ovariectomy on mammary tumor development in rats fed the CF or HF diet are shown in Chart 2 (top) and Table 2 (Group 3). Daily injection of EB to ovariectomized rats resulted in average mammary tumor diameter (Chart 1, upper panel versus Chart 2, top), and average tumor number (Table 2, Group 1 versus Group 3) approximately equal to that of sham-operated control rats fed the CF diet. Table 2 (Group 3) shows that daily EB injection into ovariectomized rats fed either a HF or CF diet produced an increase in latency period, as compared to sham-operated control rats (Group 1). The percentage of tumor incidence and average tumor number (Table 2, Group 3) were increased in the ovariectomized, EB-treated rats fed either the HF or CF diet as compared to ovariectomized rats not given EB (Table 2, Group 2). Mean tumor latency period was significantly reduced in ovariectomized rats treated with EB and fed the HF diet when compared to similarly treated rats fed the CF diet (Table 2, Group 3).

The effects of replacement with estrogen while maintaining reduced PRL levels on mammary tumor development in ovariectomized rats fed the CF or HF diet are shown in Chart 2 (bottom) and Table 2 (Group 4). Treatment of ovariectomized rats with EB and CB-154 to replace estrogen and to reduce PRL levels resulted in suppression of mammary tumor development when compared to sham-operated controls (Table 2, Group 1). Average tumor number was increased significantly in ovariectomized rats treated with EB and CB-154 and fed the HF diet, when compared to similarly treated rats fed the CF diet (Table 2, Group 4). However, no significant differences in tumor size (Chart 2, bottom), percentage of tumor incidence, and mean tumor latency period (Table 2, Group 4) were observed between ovariectomized rats treated with EB and CB-154 and fed either the HF or CF diets.

The effects of elevated serum PRL levels without estrogen replacement on mammary tumor development in ovariectomized rats fed the HF or CF diets are shown in Chart 3 (bottom) and
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Table 2 (Group 5). Elevation of serum PRL levels by daily injection of haloperidol to ovariectomized rats, without estrogen replacement, resulted in marked suppression of mammary tumor development in both CF- and HF-fed rats. Chart 3 (bottom) shows that the increase in tumor size was reduced, and Table 2 (Group 5) shows that average tumor number and percentage of tumor incidence were reduced in ovariectomized haloperidol-treated rats compared to sham-operated control rats (Group 1). No significant difference in average tumor number or increase in tumor size was observed in rats given haloperidol and fed either the HF or CF diet (Table 2, Group 5; Chart 3, bottom). An increase in percentage of tumor incidence was observed in ovariectomized haloperidol-treated rats fed the HF diet when they were compared to similarly treated rats fed the CF diet (Table 2, Group 5). However, this increase was not statistically significant (p < 0.05).

The effects of elevated serum PRL levels and replacement with estrogen on mammary tumor development in ovariectomized rats fed the CF or HF diet are shown in Chart 3 (top) and Table 2 (Group 6). By 11 weeks after DMBA administration and throughout the remainder of the experiment, ovariectomized rats fed the HF diet and given both haloperidol and EB showed a significantly greater increase in tumor size than did similarly treated rats fed the CF diet (Chart 3, top). Upon termination of the experiment, the ovariectomized rats fed the HF diet and treated with both haloperidol and EB showed an increase in average tumor number and percentage of tumor incidence (not statistically significant) and a shorter latency period for tumor appearance than did similarly treated rats fed the CF diet (Table 2, Group 6).

Serum prolactin and estradiol levels measured by RIA in blood samples collected 3, 9, and 16 weeks after DMBA administration are shown in Table 3. Prolactin levels were greatly elevated (>200 ng/ml) in rats treated with haloperidol alone or with both haloperidol and EB. CB-154 reduced serum prolactin levels markedly (<15 ng/ml) when compared to the sham-operated and EB-treated ovariectomized rats (26 to 45 ng/ml). Serum PRL levels were also reduced in ovariectomized control rats (18 to 23 ng/ml). No significant differences in serum PRL or estradiol levels were observed between similarly treated rats fed the CF or HF diet. Also, no significant differences in body weights were observed between similarly treated rats fed the CF or HF diet by 14 weeks after DMBA treatment (Table 2) or at any time during the experiment.

**DISCUSSION**

These results demonstrate that the intake of a diet containing 20% fat stimulated the development of DMBA-induced mammary tumors, even in the presence of controlled blood concentrations of PRL and estrogen, indicating that an increase in the circulating levels of either PRL or estrogen is not essential for enhancement...
of mammary tumorigenesis by a HF diet. Serum estradiol and PRL levels were regulated in ovariectomized rats by daily injections of constant doses of haloperidol, CB-154, and/or EB, but the HF diet still stimulated some parameters of mammary tumor development. Serum estradiol levels were reduced by bilateral ovariectomy and partially restored by EB administration. Serum PRL levels also were reduced by both ovariectomy and CB-154 treatment and markedly elevated by haloperidol administration. Both serum estradiol and PRL levels were not statistically different in similarly treated rats fed the HF or CF diet. When compared with similarly treated rats given either EB or haloperidol, resulting in only partial restoration of estrogen or PRL, the HF diet still enhanced some of the parameters of mammary tumorigenesis.

Therefore, the mechanism(s) by which dietary fat promotes mammary tumorigenesis do not appear to involve an increase in the circulating levels of estrogen and/or PRL.

These results indicate that maximum stimulation of mammary tumor development by a HF diet requires adequate maintenance of both estrogen and PRL levels. In ovariectomized rats given no hormone replacement, the HF diet did not stimulate any aspects of mammary tumor development, and mammary tumorigenesis was greatly reduced in these animals. Thus, estrogen and PRL are essential for mammary tumorigenesis, regardless of the amount of fat in the diet. Only when normal or elevated circulating levels of both estrogen and PRL were present, as in the sham-operated controls or in the ovariectomized rats given injections daily of haloperidol and EB, were all parameters of mammary tumorigenesis significantly increased by the HF diet.

The data present here do not eliminate the possibility that high dietary fat may promote mammary tumorigenesis by increasing the responsiveness of the incipient mammary tumor tissue to circulating levels of PRL and/or estrogen. Dunning et al. (19) reported enhanced proliferation and secretory response in mammary gland tissue from rats fed a HF diet and given diethylstilbestrol. Furthermore, Cave and Erickson-Lucas (9) reported that binding of PRL was increased in N-methyl-N-nitrosourea-induced mammary tumors in rats fed a 20.0% fat diet when compared to tumors in rats fed a 0.5% fat diet. However, observations from our laboratory (1) and the report by Ip and Ip (31) failed to show any quantitative effect of a 20% HF diet on PRL, estrogen, or progesterone receptors in DMBA-induced mammary tumors, as compared to moderate (4.5%) dietary fat levels. Differences in the mammary tumor models used and the amount of fat in the low-fat diet (0.5% versus 4.5%) may explain the discrepancies between the different laboratories.

Growth of many PRL and estrogen-independent murine mammary tumors are enhanced by HF diets (14, 21, 25, 28). Furthermore, HF dietary treatment stimulates growth of the R3230AC transplantable mammary tumor (25), which is inhibited by elevated circulating levels of PRL and/or estrogen (15, 23, 24). These reports provide further evidence that HF diets do not enhance mammary tumorigenesis by endocrine-related mechanisms.

Nonendocrine mechanisms have been proposed for the action of HF diets on mammary tumorigenesis, based primarily upon reports demonstrating increased mitotic activity in normal and neoplastic rat mammary gland cell cultures after addition of various fatty acids to the culture medium (34, 52). The fatty acid composition of mammary gland cell membranes reflects, quantitatively and qualitatively, the composition and amount of fatty acids in the diet (20, 26, 45). Such alterations in the composition of cell membranes by high polyunsaturated fat may change many membrane-associated biophysical phenomena, such as membrane fluidity, permeability, transport processes, receptor availability, intercellular communication, and/or enzyme activity, which ultimately may provide a favorable environment for high mitotic activity (3, 33). Also, increased endogenous peroxide formation due to consumption of high polyunsaturated fat diets may provide a stimulus for mammary tumor promotion by dietary fat. Benzoyl peroxide has been shown to stimulate DMBA-induced skin tumorigenesis (48). Antioxidants such as butylated hydroxytoluene reduced the stimulatory influence of HF diets on carcinogen-induced mammary tumorigenesis (35, 38). Finally, there is some evidence that HF diets may stimulate mammary tumorigenesis via mechanisms involving suppression of immune function (16, 17, 36, 41, 44, 50).

REFERENCES

21. Giovorelli, M., Podula, E., Ugazio, G., Forni, G., and Cavalla, G. Stress- and sex-linked effects of dietary polyunsaturated fatty acids on tumor growth and...


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