Influence of Hypothalamic Lesions on the Induction and Growth of Mammary Cancers in Sprague-Dawley Rats Receiving 7,12-Dimethylbenz(a)anthracene

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SUMMARY
Lesions of the median eminence of the tuber cinereum of the hypothalamus inhibit the induction of mammary cancer in Sprague-Dawley female rats that subsequently receive intragastric 7,12-dimethylbenz(a)anthracene. In contrast, such lesions significantly accelerate the growth of established mammary cancer and cancel the inhibiting effect of ovariectomy.

INTRODUCTION
Secretion of the anterior pituitary hormones are required for normal breast development and for the appearance of mammary carcinoma. The hypothalamus controls the release of most or all anterior pituitary hormones. According to McCann et al. (2), lesions in the median eminence of the tuber cinereum lead to reduction of all anterior pituitary hormones except prolactin, the release of which is enhanced.

Exploration of the influence of hypothalamic lesions upon the induction and growth of mammary carcinoma in rats appeared desirable. While our investigations were in progress, Clemens et al. (1) reported the results of their work, which was closely similar in concept and in many details to ours. They showed that placement of hypothalamic lesions before the rats were exposed to 7,12-dimethylbenz(a)anthracene (DMBA) inhibited the appearance of mammary tumors; if the hypothalamic lesions were placed 75 days following exposure to the carcinogen, the number of tumors per animal was increased. Our results are in agreement with those of Clemens et al. and are reported as an extension and confirmation of this information.

MATERIALS AND METHODS
Virgin female Sprague-Dawley rats were purchased from Holtzman Company, Madison, Wisconsin. The animals were housed singly in metal cages and received the Rockland complete rat/mouse diet and water ad libitum.

The hypothalamic lesions were placed in the median hypothalamus, using the method described by Porter (3) with slight modifications. A Kofp stereotaxic instrument was used, with insulated electrodes made from 24-gauge Nichrome V wire with an exposed flat tip. The coordinates were 0.6 mm lateral from the midline, 0.5 mm posterior to the bregma, and 0.5 mm dorsal from the base of the skull. DC current of 4 milliamperes was applied to each side for 15 seconds. The operations were performed with the rats being under anesthesia induced by phenobarbital. In the appropriate experimental groups, animals were included in the analysis of results only if on autopsy the pituitary stalk and gland were intact and if the average water intake was more than 50 ml per day. Two experiments were performed:

Experiment A. At the age of 49 days, female rats received 5 mg DMBA in 1 ml of sesame oil intragastrically via stomach tube. The dose was repeated weekly for a total of 25 mg DMBA. The animals were weighed and palpated weekly for the presence of subcutaneous masses. When one or more subcutaneous mass reached a diameter of 1 cm, the animal was assigned at random to one of 5 groups: 1, controls, no further treatment; 2, ovariectomized; 3, placement of hypothalamic lesions; 4, sham-operated; and 5, placement of hypothalamic lesions and ovariectomy. A sixth group was added to the experiment; it consisted of 15 rats that were not exposed to DMBA and had hypothalamic lesions placed at approximately the same age as the rats of Groups 2 through 5.

All animals were examined twice a week, and all subcutaneous masses were measured with external calipers. The averages of the two greatest diameters at right angles to each other were used to derive the volume of individual tumors and tumor volume per animal, during the following 3 weeks.

Experiment B. The rats, at the age of 49 days, were divided into the 4 following groups by random allocation: 1, untreated controls; 2, ovariectomized; 3, hypothalamic lesions placed; and 4, sham hypothalamic operation. One week later, all animals received a single intragastric dose of 15 mg DMBA in 1 ml sesame oil.

All animals were weighed and palpated once a week for appearance of tumors. Four weeks after the first tumor reached 0.5 x 1 cm in diameter, the animals were sacrificed. Nontumor bearers were carried for 32 weeks following the administration of DMBA.
RESULTS

Tables 1 and 2 summarize the findings of the two experiments. The effect of hypothalamic lesions on the growth of established mammary cancers is shown in Table 1. The calculated volume of tumors and the number of separate tumors per animal are significantly larger in the hypothalamic lesion group than in the control group and the group on which a sham operation was performed (Group 3 vs 1 and 4, P = 0.015). Thus, hypothalamic lesions stimulated or accelerated the growth of established mammary cancers in Sprague-Dawley rats. Ovariectomy, as is well known, drastically reduced the number and the growth of these tumors (Group 2 vs 1 and 4, P = 0.02). This ovariectomy effect appears to have been overcome by hypothalamic lesions since the group that underwent both operations was not significantly different from the controls (Group 5 vs 1 and 4, N.S.).

Production of hypothalamic lesions without exposure to DMBA did not initiate mammary tumors in any of the 15 female rats. All groups with hypothalamic lesions had significantly smaller pituitary glands at autopsy.

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial no. of rats</th>
<th>No. of rats at Day 21</th>
<th>No. of tumors per rat on Day 1</th>
<th>Total tumor volume per rat at Day 21 (cm)</th>
<th>Mean pituitary wt. (mg)</th>
<th>Mean body wt. change, Day 21 (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Controls, DMBA only</td>
<td>10</td>
<td>10</td>
<td>2.10</td>
<td>17.40 ± 6.27</td>
<td>14.90 ± 0.42</td>
<td>+26</td>
</tr>
<tr>
<td>2. DMBA + ovariectomy</td>
<td>9</td>
<td>9</td>
<td>2.33</td>
<td>4.17 ± 2.88</td>
<td>15.97 ± 1.00</td>
<td>+46</td>
</tr>
<tr>
<td>3. DMBA + hypothalamic lesions</td>
<td>14</td>
<td>11</td>
<td>2.27</td>
<td>34.13 ± 7.46</td>
<td>7.88 ± 0.95</td>
<td>+20</td>
</tr>
<tr>
<td>4. DMBA + sham lesions</td>
<td>12</td>
<td>10</td>
<td>3.20</td>
<td>11.03 ± 2.65</td>
<td>12.46 ± 0.98</td>
<td>+3</td>
</tr>
<tr>
<td>5. DMBA + hypothalamic lesions + ovariectomy</td>
<td>11</td>
<td>8</td>
<td>3.25</td>
<td>12.86 ± 7.50</td>
<td>9.01 ± 1.15</td>
<td>+23</td>
</tr>
<tr>
<td>6. Hypothalamic lesions only</td>
<td>15</td>
<td>15</td>
<td>0</td>
<td>9.25 ± 1.17</td>
<td></td>
<td>+56</td>
</tr>
</tbody>
</table>

$P \leq 0.05$ level:
1 vs 4, N.S.
1 + 4 vs 2, < 0.02.
1 + 4 vs 3, < 0.015.
1 + 4 vs 5, N.S.

Experiment A. Effect of hypothalamic lesions and ovariectomy on the growth of mammary carcinoma of rats. DMBA, 7,12-dimethylbenz(a)anthracene.

Values are mean ± standard error.

$5$ mg DMBA p.o. once weekly for $5$ weeks.

Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>Rate with carcinoma</th>
<th>MAT (weeks)$^{d,e}$</th>
<th>Mean pituitary wt. (mg)$^b$</th>
<th>Mean body wt. (gm) at Week 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Controls, DMBA only</td>
<td>16</td>
<td>13</td>
<td>81</td>
<td>11.6 ± 1.4</td>
<td>11.98 ± 0.31</td>
</tr>
<tr>
<td>2. DMBA + ovariectomy</td>
<td>15</td>
<td>1</td>
<td>7</td>
<td>14.0</td>
<td>15.04 ± 0.61</td>
</tr>
<tr>
<td>3. DMBA + hypothalamic lesions</td>
<td>12</td>
<td>3</td>
<td>25</td>
<td>26.0 ± 6.0</td>
<td>7.53 ± 0.85</td>
</tr>
<tr>
<td>4. DMBA + sham lesions</td>
<td>15</td>
<td>15</td>
<td>100</td>
<td>12.2 ± 2.1</td>
<td>11.35 ± 0.49</td>
</tr>
</tbody>
</table>

$P \leq 0.05$:
1 vs 2, < 0.01
1 vs 3, < 0.01
1 vs 4, N.S.

Experiment B. Effect of hypothalamic lesions and ovariectomy on the development of mammary adenocarcinoma with a single dose of $15$ mg 7,12-dimethylbenz(a)anthracene (DMBA) $7$ days after operations.

$^d$Mean appearance time of first adenocarcinoma after DMBA.

$^e$Values are mean ± standard error.

$^f$Tumors were excised and weighed $28$ days following detection.
Table 2 summarizes the effect of hypothalamic lesions on the induction of mammary cancers in the rat. Hypothalamic lesions are placed before DMBA is given. Here the effect is the opposite of the effect that lesions have on the growth of established tumors. The induction of tumors is significantly reduced by hypothalamic lesions (Group 3 vs 1, P < 0.01). The effect is of the same magnitude as that of ovariectomy (Group 3 vs 2, N.S.). The pituitary weight is significantly reduced by hypothalamic lesions, and the body weight is increased. On histologic examination it was found that the first tumors were adenocarcinomas of the breast with one exception, a fibroadenoma of the breast.

Thus, injury to the median hypothalamus in female Sprague-Dawley rats inhibits the induction of mammary adenocarcinomas, but it stimulates the growth of such tumors once they have been induced. These findings are in agreement with those reported by Clemens et al. (1).

**DISCUSSION**

The anterior pituitary hormones influence mammary tumor growth and development. Several hormones in combination, including adrenal and gonadal steroids, influence the structure of the mammary gland to make it susceptible or resistant to tumor development by carcinogens. Seemingly a different combination of these hormones promotes or inhibits growth of existing tumors.

Lesions in the median eminence of the hypothalamus are stated to inhibit the release of all the anterior pituitary hormone releasing factors. The lesions also destroy the site where the prolactin-inhibiting factor is released (2).

Our experiments, as those of Clemens et al., show that mammary tumor induction is inhibited when lesions are placed in the median hypothalamus before the administration of DMBA. A similar effect is produced by hypophysectomy and by ovariectomy.

In contrast with the effect on induction, hypothalamic lesions stimulate the growth of induced mammary tumors. Hypophysectomy and ovariectomy have an opposite effect, that of inhibiting the growth of such tumors. The conclusion is that prolactin plays a more important role in the growth of these tumors than during the induction phase.

In the group where hypothalamic lesions and ovariectomy were performed on animals with existing tumors, the tumor growth-promoting effect of the hypothalamic lesions and the tumor growth-inhibiting effect of ovariectomy appear to cancel each other out. This suggests a mechanism by which ovariectomy inhibits tumor growth. Progesterone, testosterone, and cortisol reduce the prolactin-inhibiting factor in the hypothalamus (4). The ovarian steroids may promote tumor growth not only by direct influence on the mammary structure, but by negative and positive feedback mechanisms in the hypothalamus.

**REFERENCES**

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