Effect of Hypothalamic Lesions on the Genesis of Spontaneous Mammary Gland Tumors in the Mouse

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SUMMARY

Continued pregnancies associated with forced breeding of intact female C3D2 F1 mice increased the incidence and reduced the latent period of development of spontaneous mammary tumors. Conversely, intact virgin mice exhibited minimal sensitivity to tumor development after a lengthy latent period. Anterior and middle hypothalamic lesions significantly altered pituitary function to the extent that a number of mice in each of these groups were rendered permanently sterile. In addition, these same lesions, although not as effective as forced breeding, did increase tumor incidence and reduce the latent period of development in nulliparous and virgin mice above the level recorded in intact virgin controls. Lesions placed in the posterior hypothalamus as well as those in the anterior and middle hypothalami that did not cause sterility were essentially ineffective in influencing tumor incidence to any greater extent than did forced breeding alone. These results indicate that enhancement of mammary tumorigenesis is related to a hormonal imbalance, neuroendocrine in origin, which probably involves the hormones prolactin and estrogen.

INTRODUCTION

Pituitary hormonal imbalance is a prime etiological factor in mammary gland tumorigenesis in mice. In view of the dominant role of the hypothalamic in regulating pituitary function, hypothalamic influence on tumor induction was investigated, as was suggested many years ago (4).

Lieberl (9) observed that gold thioglucose-induced hypothalamic lesions increased the incidence and decreased the latent period of mammary gland tumorigenesis in RIII X CBA hybrid mice. He suggested that the enhancement of mammary tumorigenesis was associated with a hormonal imbalance initiated by altered neuroendocrine mechanisms. Lacassagne and Duplan (8) similarly observed that C3H mice, when treated with reserpine, developed mammary tumors earlier and in greater numbers than did either untreated controls or treated strain XVII resistance mice. Lacassagne (6, 7) reviewed the evidence available, which indicated an intervention on the part of the hypothalamus in the pathogenesis of certain tumors of endocrine organs.

Although there is ample evidence to demonstrate tumor induction by hormonal imbalance, relatively few studies have been directed toward determining the effects of discrete areas of the central nervous system on the induction and growth of mammary gland tumors. Toh (24) and Montemurro and Toh (16) have demonstrated that hypothalamic lesions, regardless of specific location, increased the incidence and decreased the latent period of mammary tumor appearance in agent-bearing and agent-free mice. These results indicated that hypothalamic damage stimulates mammary tumorigenesis by a hormonal imbalance related to an elevated secretion of prolactin in lesioned mice. Clemens et al. (1) reported that lesions of the ME placed before DMBA treatment in rats inhibited mammary tumorigenesis. On the other hand, ME lesions placed after DMBA administration enhanced mammary tumor growth. These results have been confirmed recently (5). Similarly, Welsh et al. (25) subsequently published an account of the effects of lesions of the ME, the preoptic area of the hypothalamus, and the amygdaloid complex, on the development and growth of carcinogen-induced rat mammary tumors.

MATERIALS AND METHODS

Female mice of the C3D2 F1 (C3H/HeJ X DBA/2J) hybrid strain (Jackson Memorial Laboratories, Bar Harbor, Maine) were used in this investigation. Virgin groups were housed 5 females per cage, in force-bred groups 2 males were housed with 5 females. They were maintained in a light-controlled (12 hr/day) and temperature-controlled (72 ± 2°F) room with water and Purina Laboratory chow available ad libitum.

Hypothalamic lesions were placed with the aid of a stereotoxic device, and a refined 3-dimensional system of coordinates was adapted for use in the mouse (2, 14). The electrodes were constructed from stainless steel micropipet stylets approximately 200 μ in diameter. Mice were anesthetized with Evipan [5-(1-cyclohexen-1-yl)-1,5-dimethylbarbiturate], 0.30 mg/g body weight, administered i.p. Bilateral electrolytic lesions were placed by passing a direct anodal current of 0.5 ma intensity for 9 sec from the uninsulated tip of the electrode.

Female mice 111 to 152 days old were randomly assigned

1 The abbreviations used are: ME, median eminence; DMBA, 7,12-dimethylbenzanthracene; LFI, litter frequency index; E/D, estrus/diestrus.
to one of the following groups and treated accordingly: Group I, no treatment, force-bred; Group II, no treatment, virgin; Group III, received lesions in the anterior hypothalamic area (stereotaxic coordinates A2.75—3.8, RL0.3—0.5, H0.3—1.0). Group IV, received lesions in the middle hypothalamus in the region of the ME (stereotaxic coordinates A1.5—2.0, RL0.2, H0.3—0.5); Group V, received lesions in the posterior hypothalamus (stereotaxic coordinates A1.0, RL0.2, H0.5—1.0); Group VI, identical to Group IV above, but maintained as virgins; Group VII, operative manipulation identical to procedure used for lesioning, except that electrodes were not inserted into the brain.

Animals were examined at weekly intervals, and the body weights of all female mice were recorded. Tumors were detected by weekly palpation. Pregnancies were separated and housed individually until delivery. Litters were removed immediately or, at the latest, 24 hr postpartum to preclude suckling, and the females were returned to their original colony cages. All animals except those of Groups II and VI were force-bred in this manner. They were observed for a period of 30 weeks or more, at which time all were killed by an overdose of ether anesthesia. Mice were sacrificed only if tumor size, ulceration and bleeding, or general ill health threatened survival. Dead or moribund mice were immediately autopsied.

The cytology of exfoliated vaginal epithelium was examined during selected postoperative periods until a characteristic vaginal smear pattern could be established for the different groups. All smears were taken at approximately the same time each day 7 days a week. They were fixed with methyl alcohol, air dried, and stained with Giemsa’s solution. The evaluation of smears and the method of presentation were according to the technique described by Montemurro and Gardner (15).

**RESULTS**

All mated mice bore litters, with the exception of 9 animals with anterior (Group III) and 6 animals with middle hypothalamic lesions (Group IV); these were nulliparous for the duration of the experimental period. Because of this, Groups III and IV were each divided into 2 subgroups. Groups III-A and IV-A constituted the nulliparous mice; the multiparous mice were in Groups III-B and IV-B.

Forced breeding *per se* in intact female mice (Group I) was characterized by an increased tumor incidence and a reduced latent period of development (Table 1). Intact virgin mice, on the other hand, developed fewer tumors than force-bred mice and then only after a lengthy latent period. The parous mice with hypothalamic lesions, as well as the control mice of Group I, did not differ appreciably in either tumor incidence or latent period of development from sham-operated controls.

Nulliparous mice bearing anterior lesions and virgin mice with middle lesions had significantly greater incidences of mammary tumors from 44 to 58 weeks of age (Table 1). They also showed significantly reduced latent periods of development compared to the virgin controls (Group II). When a similar comparison is made with sham-operated controls, only Groups IV-A and VI show a significantly reduced \( p < 0.05 \) tumor incidence from 32 to 48 weeks and from 30 to 42 weeks, respectively, with a correspondingly increased latent period of development. Neither anterior- nor middle-lesioned nulliparous subgroups were significantly different in tumor incidence from their parous counterparts; however, nulliparous mice with middle hypothalamic lesions did develop tumors appreciably later than did parous mice with similar lesions. Eleven % of these multiparous mice were tumorless, whereas 33% of the nulliparous mice never developed tumors during the experimental period. Anterior and middle hypothalamic lesions were therefore a stimulus to tumor development, increasing the incidence of mammary gland tumors in nulliparous mice to levels observed in the multiparous. Most tumors were found in the 4th and 5th mammary glands, with no detectable difference in frequency. These tumors were adenocarcinomas characteristic of agent-bearing mice (21).

**The Effect of Hypothalamic Lesions on Reproductive Activity**

Recorded differences in reproductive activity were analyzed to obtain some indication of the effect of electrolytic lesions on pituitary gonadotropin and luteotropin and to correlate these effects with observed mammary tumor incidence (Table 2). All animals were initially mated within 7 days of operation, and the results include only those parous mice which developed tumors within 30 weeks of operation. As indicated earlier, 9 of 15 mice in Group III and 6 of 15 in Group IV were rendered sterile by the lesions. Furthermore, the reproductive ability of even the parous mice of these 2 groups appeared to be impaired by such lesions. Both anterior- and middle-lesioned parous mice produced fewer litters and fewer pups per mouse than did either sham-operated or force-bred controls. Although no statistically significant difference in the interval between successive deliveries could be detected, this interval, particularly in Group III-B, was longer than controls by approximately 3 days.

The LFI was calculated to express the reproductive ability of the mice with relation to their survival time (Table 2). The number of litters borne by the force-bred controls was smaller than that of the sham-operated controls. This was because they survived for only 137 ± 8 days, whereas the sham-operated controls survived for 177 ± 12 days. Thus, the LFI of the former did not differ from that of the latter (3.0 versus 3.3, respectively). On the other hand, when the reproductive patterns of the lesioned mice were expressed as a function of time, a significant inhibition of reproductive activity was observed in the parous mice with anterior lesions (Group III-B) as well as in those with middle lesions (Group IV-B).

**Target Endocrine Organ Weights**

The mean weight of the pituitary glands from sham-operated control mice was 2.6 ± 0.2 mg (Table 3). No statistically significant difference in pituitary weight was found between this group and any of the other parous groups (I, III-B, IV-B, or V). The mean weights of pituitaries removed
Effect of anterior, middle, and posterior lesions on the incidence of spontaneous mammary gland tumors and the latency of tumor development in mature C3D2 F, female mice

\[ a/b = p < 0.01; a/e = p < 0.01; c/d = p < 0.02; c/f = p < 0.05; e/f = p < 0.01; a/f = p < 0.01; n/o = p < 0.01; n/p = p < 0.01; h/i = p < 0.05; h/m = p < 0.01; k/m = p < 0.05; l/m = p < 0.05; n/q = p < 0.01. \]

Superscript "g." corresponds to time of lesion placement.

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of mice</th>
<th>Average latent period of tumor development (days postnatal)</th>
<th>No. of mice</th>
<th>Postnatal tumor incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Intact forcebred</td>
<td>14</td>
<td>227.5 ± 8.3</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>Intact virgin</td>
<td>11</td>
<td>369.4 ± 19.3^a</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>III-A</td>
<td>Anterior lesion, nulliparous</td>
<td>9</td>
<td>288.3 ± 20.4^b</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>III-B</td>
<td>Anterior lesion, parous</td>
<td>6</td>
<td>270.0 ± 13.6</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>IV-A</td>
<td>Middle lesion, nulliparous</td>
<td>4</td>
<td>326.0 ± 15.0^c</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>IV-B</td>
<td>Middle lesion, parous</td>
<td>8</td>
<td>266.1 ± 13.1^d</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>V</td>
<td>Posterior lesion</td>
<td>15</td>
<td>258.3 ± 7.5</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>VI</td>
<td>Middle lesion, virgin</td>
<td>17</td>
<td>298.6 ± 10.7</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>VII</td>
<td>Sham-operated control</td>
<td>18</td>
<td>250.9 ± 14.3</td>
<td>20</td>
<td>0</td>
</tr>
</tbody>
</table>

^a Mean ± S. E.

### Table 2

**Effect of anterior, middle, and posterior lesions on reproductive activity of tumor-bearing female C3D2 F, mice within 30 weeks of operation**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of mice</th>
<th>Litters</th>
<th>Pups/mouse</th>
<th>Pups/litter</th>
<th>Days between deliveries</th>
<th>Litters at first tumor appearance</th>
<th>Survival time (days postoperatively)</th>
<th>LFI</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Intact forcebred</td>
<td>14</td>
<td>4.2 ± 0.4^b</td>
<td>24.6 ± 2.4</td>
<td>6.0 ± 0.4</td>
<td>26.5 ± 1.0</td>
<td>2.6 ± 0.3</td>
<td>137.1 ± 8.0</td>
<td>3.0 ± 0.1</td>
</tr>
<tr>
<td>III-B</td>
<td>Anterior lesion, parous</td>
<td>6</td>
<td>3.0 ± 0.7</td>
<td>16.3 ± 3.7</td>
<td>5.8 ± 0.5</td>
<td>30.7 ± 2.5</td>
<td>2.3 ± 0.5</td>
<td>153.7 ± 12.6</td>
<td>2.1 ± 0.5</td>
</tr>
<tr>
<td>IV-B</td>
<td>Middle lesion, parous</td>
<td>8</td>
<td>4.1 ± 0.8</td>
<td>18.9 ± 5.0</td>
<td>4.3 ± 0.3</td>
<td>28.1 ± 2.2</td>
<td>3.3 ± 0.4</td>
<td>172.4 ± 16.8</td>
<td>3.4 ± 0.4</td>
</tr>
<tr>
<td>V</td>
<td>Posterior lesion</td>
<td>15</td>
<td>5.5 ± 0.5</td>
<td>31.9 ± 2.7</td>
<td>6.1 ± 0.3</td>
<td>26.3 ± 1.2</td>
<td>3.7 ± 0.4</td>
<td>164.5 ± 11.6</td>
<td>3.3 ± 0.2</td>
</tr>
<tr>
<td>VII</td>
<td>Sham-operated control</td>
<td>16</td>
<td>5.5 ± 0.4</td>
<td>40.7 ± 3.0</td>
<td>7.6 ± 0.3</td>
<td>27.8 ± 1.2</td>
<td>3.8 ± 0.4</td>
<td>176.7 ± 11.9</td>
<td>3.3 ± 0.1</td>
</tr>
</tbody>
</table>

^a LFI, no. of litters/survival time (days postoperatively) X 100.

^b S.E.

^c p compared with sham-operated control (Group VII).

### Table 3

**Endocrine organ weights of mature tumor-bearing intact and hypothalamic-lesioned female C3D2 F, mice**

\[ b/c = p < 0.02; \quad b/d = p < 0.02; \quad b/f = p < 0.01; \quad d/g = p < 0.02; \quad f/g = p < 0.01; \quad e/f = p < 0.01; \quad h/i = p < 0.01; \quad h/j = p < 0.01; \quad k = p < 0.02. \]

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment^a</th>
<th>No. of mice</th>
<th>Average ovarian weight (mg)</th>
<th>Average pituitary weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Intact forcebred</td>
<td>12</td>
<td>14.0 ± 1.6</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>II</td>
<td>Intact virgin</td>
<td>11</td>
<td>17.9 ± 1.5^b</td>
<td>2.6 ± 0.2^h</td>
</tr>
<tr>
<td>III-A</td>
<td>Anterior lesion, nulliparous</td>
<td>9</td>
<td>12.3 ± 1.5^c</td>
<td>3.0 ± 0.1</td>
</tr>
<tr>
<td>III-B</td>
<td>Anterior lesion, parous</td>
<td>5</td>
<td>14.1 ± 1.7</td>
<td>2.6 ± 0.3</td>
</tr>
<tr>
<td>IV-A</td>
<td>Middle lesion, nulliparous</td>
<td>2</td>
<td>6.6 ± 2.9^d</td>
<td>1.4 ± 0.2^l</td>
</tr>
<tr>
<td>IV-B</td>
<td>Middle lesion, parous</td>
<td>7</td>
<td>13.5 ± 1.9^e</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>V</td>
<td>Posterior lesion</td>
<td>11</td>
<td>16.1 ± 1.5</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>VI</td>
<td>Middle lesion, virgin</td>
<td>17</td>
<td>6.9 ± 1.3^f</td>
<td>2.0 ± 0.1^l</td>
</tr>
<tr>
<td>VII</td>
<td>Sham-operated control</td>
<td>17</td>
<td>14.7 ± 1.0^g</td>
<td>2.6 ± 0.2^k</td>
</tr>
</tbody>
</table>

^a All mice were sacrificed 30 weeks after placement of lesions.

^b S.E.
from middle-lesioned nulliparous and virgin mice with similar lesions were 1.4 ± 0.2 and 2.0 ± 0.1 mg, respectively. These values were both significantly smaller than that of intact virgins, with only the latter being also smaller than that of sham-operated controls. The pituitaries of nulliparous anterior-lesioned mice were slightly heavier than either control group.

The ovaries of both Group IV-A and Group VI were significantly smaller than those of either intact virgin or sham-operated controls. The ovaries of both these groups were smaller than those of their lesioned parous counterparts (Group IV-B) as well. The mean weight of the ovaries of virgin control mice was larger than any other group; many of these ovaries were cystic.

**Histology**

**Ovaries.** The ovaries of the nulliparous mice with anterior hypothalamic lesions show an abundance of well-developed primary and secondary follicles indicative of follicular stimulation and few, if any, corpora lutea (Fig. 1). The mean weight of the ovaries of these mice was 12.3 ± 1.5 mg. The parous mice with anterior hypothalamic lesions, on the other hand, had a slightly greater ovarian weight (14.1 ± 1.7 mg), and microscopically their ovaries contained numerous follicles in various stages of maturation, and ovulation and well-formed corpora lutea (Fig. 2). Most of the ovaries of the nulliparous mice with middle hypothalamic lesions were atrophic, weighing 6.6 ± 2.9 mg, and contained few follicles and few corpora lutea with an abundance of what appeared to be corpora albicains within the interstitium (Fig. 3); however, in some cases, large primary and secondary follicles and corpora lutea were observed. The ovaries of middle hypothalamic-lesioned virgin mice were similar in weight (6.9 ± 1.3 mg) and histological appearance to the nulliparous mice of Group IV (Fig. 4). The ovaries of parous mice with middle hypothalamic lesions, on the other hand, were slightly larger (13.5 ± 1.9 mg) and histologically resembled those of cycling mice, with numerous mature follicles and well-developed corpora lutea (Fig. 5).

**Mammary Glands.** Whole-mount preparations of the mammary glands of virgin control mice revealed moderate ductal development, the presence of many lateral buds along the main ducts, and small clusters of alveoli along the sides and ends of ducts (Fig. 6). The nulliparous subgroup of mice with anterior hypothalamic lesions had a greater degree of ductal development than did virgin controls, with small to large clusters of alveoli (Fig. 7). Mammary glands of middle hypothalamic-lesioned virgin mice and nulliparous mice with middle hypothalamic lesions were similar in appearance, exhibiting a poor degree of ductal development with very few lateral buds and clusters of alveoli (Figs. 8 and 9). Whole mounts of the mammary glands of normal cycling multiparous animals are not included because of the variation in their structure relative to the time of pregnancy.

**Vaginal Smear Patterns**

Vaginal cycles of 2 typical mice from Groups II, III, IV, and VI are shown in Chart 1 and a quantitative analysis of the exfoliative vaginal cytology of all animals studied is provided in Table 4. The frequency of estrus and diestrus was determined as follows: vaginal histories were designated as estrus if the smears fell between P1 or P2 and M1 and as diestrus if they fell between M1 or M2 and P1, inclusively. Among the virgin controls, 31% of the total smears taken were estrus, and 69% were diestrus. Similar patterns of vaginal cycles were observed in middle hypothalamic-lesioned animals of Groups IV-A and VI. Among these animals, 12 to 15% of the smears were estrus; 85 to 89% were diestrus, and the E/D ratios were among the lowest recorded. Diestrus occurred with almost twice the frequency of estrus in virgin controls. Mice with anterior hypothalamic lesions, as expected, showed the highest E/D ratio, which is reminiscent of the hyperestrogenization following such lesions in the rat. The E/D ratio in the initial 23-day period was 2.44. In the subsequent 36-day period, the ratio changed to 0.27 because of the increased frequency of diestrus. This phenomenon was found only in those mice with anterior hypothalamic lesions and was not related to advancing age.

**Location of Electrolytic Lesions**

Serial sections of all brains were examined microscopically. The size of the lesions was approximately 0.3 x 0.5 mm at 30 weeks after placement. Most lesions were found to be bilaterally symmetrical.

**Anterior Hypothalamic Lesions.** These were situated in the medial preoptic and anterior hypothalamic areas (Fig. 10). They began at the anterior level of the optic chiasm and proceeded caudally to the anterior aspect of the arcuate and ventromedial nuclei. They were restricted for the most part to the medial preoptic, suprachiasmatic, and ventral paraventricular nuclei, the anterior hypothalamic area and the periventricular gray. Among the nulliparous animals in this group, the lesions extended further caudally into the arcuate and ventromedial area around the infundibular recess.

**Middle Hypothalamic Lesions (Groups IV and VI).** These were located in the region of the median eminence of the tuber cinereum. The ventromedial nucleus, arcuate nucleus, and ME were invariably involved (Fig. 11).

**Posterior Hypothalamic Lesions.** These extended from the caudal infundibular region to the mammillary bodies (Fig. 12). They invariably involved the caudal arcuate, the ventral premamillary, and much of the mammillary nuclear complex.

**DISCUSSION**

The results of this investigation indicate that the continued pregnancies associated with forced breeding in intact female mice accelerated the appearance of spontaneous mammary tumors. Conversely, intact virgin mice exhibited minimal sensitivity to tumor development and then only after a lengthy latent period. These observations confirm earlier reports by Muhlböck (17, 18), Marchant (10, 11), and others. Anterior and middle hypothalamic lesions altered pituitary trophic function to the extent that 9 mice in the former group and 6 mice in the latter were rendered permanently sterile. In
addition, these same lesions, although not as effective as forced breeding *per se* did enhance the tumor incidence in lesioned nulliparous and lesioned virgin mice over and above the level recorded in intact virgin controls. Lesions situated in the posterior hypothalamus, as well as those lesions in the anterior and middle hypothalamus that did not cause sterility, were essentially ineffective in influencing the tumor incidence to any greater extent than did forced breeding alone (Table 1).

Toh (24) and Montemurro and Toh (16) reported that hypothalamic lesions, regardless of specific location, increased

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Table 4

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of mice</th>
<th>Estrus (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Diestrus (%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Average E/D ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>Intact virgin</td>
<td>5</td>
<td>31</td>
<td>69</td>
<td>0.47</td>
</tr>
<tr>
<td>III-A</td>
<td>Anterior lesion, nulliparous</td>
<td>8</td>
<td>59</td>
<td>42</td>
<td>2.44</td>
</tr>
<tr>
<td></td>
<td>Initial 23-day period</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Subsequent 36-day period</td>
<td>8</td>
<td>18</td>
<td>83</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>Combined</td>
<td>8</td>
<td>36</td>
<td>64</td>
<td>0.64</td>
</tr>
<tr>
<td>IV-A</td>
<td>Middle-lesion, nulliparous</td>
<td>6</td>
<td>12</td>
<td>89</td>
<td>0.14</td>
</tr>
<tr>
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<td>Middle-lesion, virgin</td>
<td>18</td>
<td>15</td>
<td>85</td>
<td>0.24</td>
</tr>
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</table>

<sup>a</sup> Period extending from P₁ or P₂ to M₁ inclusively.

<sup>b</sup> Period extending from M₁ or M₂ to P₁ inclusively.
Hypothalamic Lesions and Mammary Tumorigenesis

the incidence and decreased the latent period of tumor development in both agent-bearing C3D2 F1 and agent-free CAF1 female mice. They concluded that hypothalamic damage stimulated mammary tumorigenesis by a hormonal imbalance related to an elevated secretion of prolactin. In a recent study (26), spontaneous mammary tumor incidence and blood prolactin levels were significantly increased following ME lesions in multiparous rats. The results of the present study differ from these reports in the following respects: (a) no significant enhancement of tumor incidence or decrease in latent period of development was observed in multiparous mice bearing either anterior, middle, or posterior hypothalamic lesions; and (b) unlike the previous reports (16, 24), we were able to observe only a stimulation of the mammary tumorigenic process by hypothalamic lesions in virgin mice and in those mice rendered sterile by lesions in the anterior and middle hypothalamus. Unfortunately, we are unable to provide a satisfactory explanation at this time for the apparent discrepancies in results emanating from the one laboratory.

In the mouse, the effect if any of hypothalamic damage on the development of mammary tumors is negligible when superimposed on a background of multiparity. Table 1 indicates that the action of such lesions may even be inhibitory when the comparison is made to the force-bred control group. However, the extent to which hypothalamic damage per se affects mammary tumor development in the absence of the multiple pregnancies of forced breeding is difficult to ascertain unequivocally. This maneuver certainly increases the tumor incidence over and above that of unoperated virgins (Table 1), but it never reaches the level seen in force-bred controls. No doubt the elevated tumor incidence in nulliparous mice is related to the hormonal imbalances induced by the lesions, which is responsible for their sterility in the first place. The 9 nulliparous mice bearing anterior hypothalamic lesions (Group III-A) showed all the signs of hyperestrogenization observed following such lesions in the rat (3). Their ovaries, although not excessively large, were characterized by many primary and secondary follicles but no corpora lutea (Fig. 1); their mammary glands showed marked proliferation of ductal elements characteristic of estrogen stimulation (Fig. 7). The vaginal cycles of these mice were predominantly estrus for many weeks after operation (Chart 1, Table 4); the mean pituitary weight of these animals was the largest of all the groups studied (Table 3). Since high levels of estrogen, either of endogenous or exogenous origin, have been implicated repeatedly in the development of mammary gland tumors in mice (19, 23), it is likely that the hyperestrogenization of the mice with anterior hypothalamic lesions is the cause of their significant increase in tumor incidence. However, it is also well established that estrogen can stimulate the synthesis and release of prolactin from the pituitary (20, 22). This may explain the abrupt reversion from predominantly estrus to predominantly diestrus at about 85 days postoperatively in the anterior-lesioned mice (Chart 1) and so contribute to the tumorigenic stimulus provided by the elevated levels of estrogen.

The increased tumor incidence in virgin mice with middle hypothalamic lesions (Group VI) and in the 6 nulliparous mice with similar lesions (Group IV-A) is less easily explained (Table 1). Lesions placed in the ME have been reported to impair the release of prolactin-inhibiting factor (12), enhance the release of prolactin, and reduce the secretion of all other pituitary trophic hormones (13). Welsch et al. (25) reported extensive mammary gland growth comparable to that of late pregnancy in virgin rats 10 and 25 days following placement of ME lesions. Clemens et al. (1) and Klaiber et al. (5) attributed the elevated tumor incidence in DMBA-treated rats bearing ME lesions to an increase in the circulating levels of prolactin. The presence of adequate levels of ovarian steroids was found to be essential for this response to hypothalamic lesions. Our data are not entirely consistent with these reports. Although we observed an increase in tumor incidence following middle hypothalamic lesions (Table 1), we saw no evidence of abnormally high levels of prolactin in these animals. The mean weight of the ovaries and the pituitary glands were the smallest of all the animals studied (Table 1), the ovaries were atrophic (Figs. 3 and 4), and the adrenal glands at autopsy were the smallest of all the mice studied. (6.3 ± 1.6 and 5.9 ± 0.7 mg, respectively). Their mammary glands were composed almost solely of ducts with few if any end buds or alveoli (Figs. 8 and 9), quite unlike those subjected to high levels of circulating prolactin (15, 26). We do not know whether the middle hypothalamic lesions in our mice produced an initial and transitory elevation of pituitary prolactin secretion which might account for their slightly higher ultimate tumor incidence. We do know, however, that at the time of autopsy, some 30 to 40 weeks after hypothalamic lesioning, these mice presented a typical picture of panhypopituitarism.

The results of these experiments point clearly to the fact that all of the hormonal changes in the milieux interieur those that accompany pregnancy represent the strongest stimuli to the mammary tumorigenic process in the mouse. Hyperestrogenization, and possible hypersecretion of prolactin, which follows destruction of the anterior hypothalamus, stimulates mammary tumorigenesis in mice rendered sterile by this procedure. The elevated levels of either hormone would effectively increase tumor incidence; however, the combined or synergistic action resulting from elevated titers of both hormones appears to be most effective.

ACKNOWLEDGMENTS

We gratefully acknowledge the technical assistance of Mr. R. Blackman.

REFERENCES

Figs. 1 to 5. Photomicrographs of representative ovaries. H & E, x 25 to 30.

Fig. 1. Ovary from a 366-day-old nulliparous mouse (Group III-A) with lesions in the anterior hypothalamus, showing many primary and secondary follicles and no large corpora lutea. The combined weight of both ovaries was 10.6 mg.

Fig. 2. Ovary from a 329-day-old parous mouse (Group III-B) with lesions in the anterior hypothalamus, showing numerous follicles in various stages of maturation and well-developed corpora lutea. The combined weight of both ovaries was 18.6 mg.

Fig. 3. Ovary from a 397-day-old nulliparous mouse (Group IV-A) with lesions in the middle hypothalamus, showing few follicles and corpora lutea, degenerative changes, and what appear to be remnants of corpora lutea within the interstitium. The combined weight of both ovaries was 3.7 mg.

Fig. 4. Both ovaries from a 344-day-old virgin mouse (Group VI) with lesions in the middle hypothalamus. They are histologically similar to those found in Group IV-A (Fig. 3). The combined weight of both ovaries was 2.7 mg.

Fig. 5. Ovary from a 315-day-old parous mouse (Group IV-B) with lesions in the middle hypothalamus, showing many corpora lutea and several maturing follicles. The combined weight of both ovaries was 22.0 mg.

Figs. 6 to 9. Representative whole mounts of thoracic mammary glands. Mayer’s hematoxylin, X 16 to 20.

Fig. 6. Mammary gland from a 383-day-old virgin mouse (Group II), showing moderate ductal development with small clusters of alveoli along the sides and ends of the ducts. Duct width and branching was small, as was the overall area covered by the mammary gland parenchyma.

Fig. 7. Mammary gland from a 337-day-old nulliparous mouse (Group III-A) with lesions in the anterior hypothalamus, showing greater degree of ductal development than virgin controls with small to large clusters of alveoli.

Fig. 8. Mammary gland from a 401-day-old nulliparous mouse (Group IV-A) with lesions in the middle hypothalamus. It is morphologically similar to the mammary glands of Group VI described below.

Fig. 9. Mammary gland from a 340-day-old virgin mouse (Group VI) with lesions in the middle hypothalamus, showing poor ductal development with few or no alveoli.

Figs. 10 to 12. Representative photomicrographs of frontal sections through the hypothalami of the mouse brain. F, fornix; IR, infundibular recess; MC, mammillary complex; OT, optic tract; SN, substantia nigra; THAL, thalamus; IC, internal capsule; III, 3rd ventricle. Arrows, lesions. Weigert-Weil (Lillie’s Variant), X 20.

Fig. 10. Frontal section through the hypothalamus of a parous female mouse (Group III-B) at the level of the caudal chiasm, showing the location of the anterior hypothalamic lesions placed with coordinates A3.0, RL0.5, and H0.5.

Fig. 11. Frontal section through the hypothalamus of a virgin mouse (Group VI) at the level of the infundibulum, showing the location of the middle hypothalamic lesions placed with the coordinates A2.0, RL0.2, and H0.3.

Fig. 12. Frontal section through the posterior hypothalamus of a parous female mouse (Group V) at the level of the mammillary complex, showing the location of posterior hypothalamic lesions placed with the coordinates A1.0, RL0.2, and H0.5.
Effect of Hypothalamic Lesions on the Genesis of Spontaneous Mammary Gland Tumors in the Mouse

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