Increased Mammary Gland Response to Pituitary Mammotrophic Hormones by Estrogen in Rats

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Synopsis

The local effects of estradiol benzoate (EB) on mammary growth were investigated. The pellet of EB mixed with cholesterol at several ratios was implanted in the fat pad of the right third thoracic gland of the ovariectomized 3-month-old Sprague-Dawley female rat. The contralateral gland was served as the control. All the rats given EB pellets showed estrous smears for several days and subsequently continued diestrous smears. Seven-10 days after the onset of diestrus, some rats were killed by decapitation. The other rats were further grafted with the isologous pituitaries under the right kidney capsule and were killed after 20 days. By the observation of the whole mount preparation and the determination of mammary DNA, the mammary gland was found to be promoted in the growth only around the implanted pellet in the rat bearing both EB pellet at the ratio of 1:5,000 and grafted of 2 pituitaries. On the other hand, at all dose levels examined, EB implant alone did not induce mammary growth. These results indicate that EB increases the mammary response to pituitary mammotrophic hormones even in its amount which did not promote by itself the mammary growth at all, and provide evidence that one of the actions of estrogen on mammary growth is to sensitize the gland to pituitary mammotrophic hormones.

The pituitary and ovarian hormones are the most important regulators of normal mammary growth. Since ovarian hormones have little or no ability to promote mammary growth in the absence of the anterior pituitary (Lyons et al., 1958; Meites and Hopkins, 1961), whereas anterior pituitary hormones are effective in the absence of the steroid hormones (Clifton and Furth, 1960; Talwalker and Meites, 1961, 1964; Dao and Gawlak, 1963; Meites and Kragt, 1964), it can be concluded that the anterior pituitary hormones are the primary stimulators of mammary growth (Meites, 1966).

The gonadal steroids are considered to participate in mammary growth both indirectly and directly. Estrogen acting indirectly stimulates the secretion of the pituitary mammotrophic hormones. Its direct actions are possible to sensitize the mammary tissue to the pituitary mammotrophic hormones and to stimulate by itself directly the mammary growth. Several investigators have found the direct stimulation of estrogen on mammary growth by the local application of estrogen (Lyons and Sako, 1940; Chamberlin et al., 1941; Nelson, 1941). However, in these experiments, the control gland of the contralateral side also grew in some cases, although in less degree. These indicate that the administered estrogen acts systemically as well as locally. The phenomena observed in their experiments were the collective results of the actions of pituitary mammotrophic hormones stimulated the secretion by estrogen and of possible two local actions of estrogen.

Received for publication February 12, 1971.
on mammary glands. Therefore, these experiments have not determined whether estrogen stimulated the mammary growth directly, or sensitized the gland to pituitary mammotrophic hormones or both. The present experiment was attempted to clarify this point by designing such condition of estrogen as to act only locally.

Materials and Methods

Ovariectomized, 3-month-old female Sprague-Dawley rats bred in our laboratory were used. They were maintained in an air-conditioned and artificially illuminated room and provided commercial diet and water ad libitum. Ovariectomy was performed approximately 20 days before the implantation of pellet of estrogen (estradiol benzoate: EB, 1 µg = 10 IU. Nutritional Biochemical Corp., Cleveland, Ohio). EB was thoroughly mixed with cholesterol at several different ratios as shown in Table 1, pelleted 5 mm in diameter, 2 mm in thickness and 50 mg in weight, and implanted in the central part of the fat pad of the right third thoracic gland. The contralateral gland was served as the control. Vaginal smear was made once a day throughout the experiment beginning 3 weeks before the pellet implantation. Vaginal smear was chosen as the index whether EB implant acted only locally, because it was proved to be the most sensitive measure among several behavioral and somatic response to estrogen (Davidson et al., 1968). The smears of all the rats given EB implants showed the temporary cornification for several days and subsequently the constant diestrous features returned, which would be due to the coating by the connective tissue of the pellet and would indicate that EB acted no longer systemically after this time.

In Experiment I, the rats were implanted EB pellets only at the ratios of 1:5,000 to 1:40,000 and killed by decapitation 7–10 days after onset of diestrus. In Experiment II, the rats with EB pellets (1:5,000 or 1:10,000) were further given graft of 1 or 2 isologous pituitaries each under the right kidney capsules 7–10 days after the onset of diestrus and killed 20 days after pituitary grafting. Bilateral thoracic third glands were removed and used for whole mount preparations in Experiments I and II. The mammary growth was examined under 10 fold magnification. In Experiment III, the rats with EB pellet implants (1:5,000) were divided into two groups 7–10 days after the onset of diestrus; one was killed immediately and another was further grafted with 2 pituitaries and killed after 20 days. The mammary DNA contents of the bilateral glands were determined separately. A portion of the fat pad around the pellet about (1.5 × 1.5) cm² in area and that of the corresponding site were removed from the experimental and control glands, respectively. The dry fat free tissue (DFFT) treated with hot alcohol and ether was used for the determination of DNA. DNA was extracted by the procedure of Schneider (1944), assayed by diphenylamine reaction of Dische (1930) and expressed as µg per 100 mg DFFT. The comparison between the experimental and control glands was performed in each rat.

Results and Discussion

The results are illustrated in Table 1. In Experiment I, no difference existed between the experimental and control sides of the glands in each rat irrespective of the dose levels of EB examined. All the glands showed only the rudimentary duct systems with regressed end-buds quite similarly to the glands of rats with the pellets of cholesterol only. In Experiment II, the additional grafting of 2 isologous pituitaries each induced the marked mammary growth, especially lobulo-alveolar development, only around the pellet implants in all the rats bearing EB pellets of 1:5,000 (Fig. 1–A), whereas the contralateral glands in the same rats remained almost rudimentary (Fig. 1–B). The local enhancement by EB of mammary response to pituitary mammotrophic hormones was further confirmed quantitatively by the assay of mammary DNA content in Experiment III. Mammary DNA was significantly higher in the experimental gland than in the contralateral control gland in this group (P < 0.05), while there was little difference between the experimental and control glands in this measure in the other two groups. These results indicate that the implanted EB increases the mammary response to pituitary mammotrophic hormones, which causes the local mammary growth, and they have demonstrated that one of the actions of estrogen on mammary growth is to sensitize the gland to pituitary mammotrophic hormones.
Fig. 1. The representative whole mount preparations of mammary glands of the ovariectomized rat given both pellet of estradiol benzoate (EB) at the ratio of 1:5,000 and graft of 2 isologous pituitaries. EB was implanted in the central part of the fat pad of the right third thoracic gland and pituitaries were grafted under the right kidney capsule.

A: Experimental gland: Mammary ducts and lobulo-alveoli develop well only around the EB pellet implant.

B: Contralateral gland: The mammary development is much less than in the experimental gland.

Table 1. Effects of pellet implant of estradiol benzoate (EB) on mammary growth of ovariectomized rats

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>Ratio of EB to cholesterol (amount of EB)</th>
<th>No. of rats</th>
<th>Duration of vaginal cornification (day)</th>
<th>Time of sacrifice</th>
<th>Index of mammary growth</th>
<th>Mammary gland</th>
<th>Difference between experimental and control glands</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0 (Cholesterol only)</td>
<td>5</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1:5,000 (10μg)</td>
<td>9</td>
<td>9.1 ± 1.6</td>
<td>7–10 days after the onset of diestrus</td>
<td>Structure</td>
<td>Only rudimentary duct system with regressed end buds</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1:10,000 (5μg)</td>
<td>5</td>
<td>4.8 ± 0.5</td>
<td></td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1:20,000 (1μg)</td>
<td>5</td>
<td>1.2 ± 0.5</td>
<td></td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1:40,000 (0.5μg)</td>
<td>5</td>
<td>1.0 ± 0.3</td>
<td></td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>1:5,000 + 1AP</td>
<td>6</td>
<td>7.8 ± 1.3</td>
<td>20 days after AP grafting which was performed 7–10 days after the onset of diestrus</td>
<td>Structure</td>
<td>A little better than Exp. I</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1:10,000 + 1AP</td>
<td>5</td>
<td>4.0 ± 0.3</td>
<td></td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1:5,000 + 2AP</td>
<td>5</td>
<td>8.2 ± 1.0</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>III</td>
<td>0 (Cholesterol only)</td>
<td>6</td>
<td>0</td>
<td>Same as Exp. I</td>
<td>DNA</td>
<td></td>
<td>-51~267 (ns)4)</td>
</tr>
<tr>
<td></td>
<td>1:5,000</td>
<td>7</td>
<td>9.2 ± 1.8</td>
<td></td>
<td></td>
<td></td>
<td>57~329 (ns)</td>
</tr>
<tr>
<td></td>
<td>1:5,000 + 2AP</td>
<td>7</td>
<td>7.3 ± 0.6</td>
<td>Same as Exp. II</td>
<td></td>
<td></td>
<td>2~148 (*)</td>
</tr>
</tbody>
</table>

1) Pellet was implanted in the central part of the fat pad of the right thoracic third gland.
2) AP: Anterior pituitary, which was grafted under the right kidney capsule.
3) Mean ± S.E.M.
4) Range in difference (μg) (= experimental – control) at P=0.05. ns P > 0.1, *P < 0.05.
Although the present results do not always exclude another possibility of the action of estrogen, the direct stimulation of mammary growth, the sensitizing action is proved more prominent, because EB increased the mammary response to pituitary mammtotropic hormones in its amount which did not promote by itself the mammary growth at all.

No effect of EB pellet implant on local mammary growth was observed in the rats with both graft of 1 pituitary each and EB implant of 1:5,000 or 1:10,000, although the slight regeneration of mammary gland by pituitary graft was found in the bilateral glands. This is probably due to the insufficient amounts of EB and/or pituitary mammtropic hormones for detection by the present techniques of local effects.

There were no differences in the anterior pituitary weights among groups in all experiments. The uterine weights were rather heavier in the rats with EB pellet implants than in the rats with cholesterol pellet implants. This would be ascribed to the after-effect of EB when it acted systemically, because the uterine weight declined drastically for a while after the cessation of estrogen administration, and subsequently the decreasing rate became much slower (Ota, personal communication).

Acknowledgment

Our thanks are due to Dr. J. Mori, National Institute of Animal Industry, for his valuable comments on this work.

References