Note

Inhibitory Effect of Estrogen on Mammary Growth and Its Counteraction by Pituitary Isograft in Mice

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Synopsis

Mammary growth was markedly inhibited in mice by a single subcutaneous injection of 100 µg hexestrol dicaprylate despite the fact that the pituitary prolactin levels were significantly higher in these mice than in the intact controls. The degeneration of mammary gland was completely counteracted by the simultaneous isologous pituitary grafting, indicating that the additional supply of prolactin from the grafts overcame the inhibitory action of estrogen on mammary growth. These results have demonstrated the existence of antagonistic effects between prolactin and estrogen on mammary growth in mice at some dose levels of estrogen.

In a series of experiments on the pharmacological and physiological roles of several hexestrol derivatives in the pituitary and mammary gland functions, we were aware that mammary growth of mice was markedly inhibited by hexestrol dicaprylate despite the fact that pituitary prolactin levels of these estrogentreated mice were extremely high. Prolactin is evidenced to be the principal mammogenic hormone in mice (Nandi, 1959; Yanai and Nagasawa, 1971a). While estrogen is another mammogenic hormone, it is well known to suppress, in some cases, lactation (Smith, 1959; Mizuno et al., 1968; Ōta, 1968) and hormone responsive mammary tumor growth (Grenn et al., 1960; Gropper and Shimkin, 1967; Teller, 1969). Therefore, it would be suggested that there existed some antagonistic effects between prolactin and estrogen on mammary growth of mice at some dose levels of estrogen. The present experiment was performed to clarify this point.

Materials and Methods

Eighty to 90-day-old C3H/He virgin mice weighing 21–23 g were used. They were divided into 3 groups: Group I was not treated and served as the intact control; group II was given a single subcutaneous injection of 100 µg hexestrol dicaprylate (Takeda Chemical Industries, Ltd., Tokyo) dissolved in 0.1 ml sesame oil which was found to be a long acting estrogenic dose (Nagasawa and Yanai, unpublished); group III was given both 100 µg hexestrol dicaprylate and isologous pituitary grafts under the bilateral kidney capsules two each. Throughout the experiment, they were maintained in an air-conditioned and artificially illuminated room and offered commercial diet and water ad libitum. Vaginal smears were made every morning beginning 2 weeks before estrogen injection. Four weeks later, they were killed by decapitation. Anterior pituitary was rapidly removed, weighed and kept at −20°C in groups I and II for prolactin assay by disc electrophoresis on 10% polyacrylamide gel (Cheever et al., 1969; Yanai and Nagasawa, 1971b). Pituitary prolactin secretory activity was estimated from the pituitary levels of the hormone. The right thoracic mammary gland was fixed in Bouin’s fluid for the whole mount evaluation. The degree of mammary growth was rated from 1 to 7 in increments of 1 by the modified standard of Wrenn et al. (1966).

Received for publication September 25, 1971.
Results

The results are illustrated in Table 1. There were no differences among groups in either body weight at sacrifice or anterior pituitary weight. Both the pituitary prolactin content and concentration were significantly higher in group II than in group I (P < 0.01). Groups I and III were significantly higher than group II in the mammary rating (P < 0.01) and group III was also higher than group I in this measure (P < 0.01). While the mammary gland of group I showed the well developed duct system with numerous end-buds (Fig. 1-1), the gland of group II was mainly composed of duct system and the end-buds were mostly degenerated (Fig. 1-2). On the other hand, numerous lobulo-alveoli were seen and some alveoli contained milk-like substance in the lumina in group III (Fig. 1-3). Group I had regular estrous cycles of 4 or 5 days accompanied with relatively long diestrous periods similar to those often observed in normal female C3H/He mice (Nagasawa et al., 1967a). Group II showed the continued estrous smears throughout the experiment beginning a few days after estrogen injection. Group III showed the continued diestrus during the experiment, although a few mice had one estrus each at about 15 days’ interval.

Discussion

The present results provide ample evidence that a single subcutaneous injection of hexestrol dicaprylate markedly suppresses the mammary growth in mice. Estrogen inhibits lactation and mammary tumor growth in some conditions and is often used for the clinical purpose. Although the mechanism of its inhibitory role in mammary gland is not understood yet, it is clear that inhibition by estrogen of normal or neoplastic mammary growth and lactation is not via its suppression of pituitary prolactin secretion. The serum prolactin levels of the ovariectomized, carcinogen-treated rats given a continuously large dose of estrogen were extremely high, whereas the mammary tumor incidence was suppressed and its growth was completely inhibited in these rats (Nagasawa and Meites, unpublished). Chen and Meites (1970) also reported in ovariectomized rats that estrogen stimulated the pituitary prolactin secretion even in its high dose levels. Moreover, in the present experiment, the pituitary prolactin levels were significantly higher in hexestrol dicaprylate treated mice than in the controls. All the results would lead us to infer that the effect of estrogen on mammary gland was based on its dual participations at some dose levels; it

Table 1. Body weight, mammary growth and anterior pituitary (AP) prolactin levels in each group

<table>
<thead>
<tr>
<th>Group and treatment</th>
<th>No. of mice</th>
<th>Body wt. at sacrifice (g)</th>
<th>AP weight (mg)</th>
<th>AP prolactin level (Optical density*)</th>
<th>Mammary rating</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>/AP</td>
<td>mg AP</td>
</tr>
<tr>
<td>I. Control</td>
<td>20</td>
<td>24.8 ± 0.6</td>
<td>2.00 ± 0.10</td>
<td>23.2 ± 1.3a</td>
<td>11.6 ± 0.7c</td>
</tr>
<tr>
<td>II. Hexestrol</td>
<td>15</td>
<td>24.1 ± 0.5</td>
<td>2.06 ± 0.09</td>
<td>37.8 ± 2.0b</td>
<td>18.2 ± 1.0d</td>
</tr>
<tr>
<td>dicaprylate 100 µg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III. Hexestrol</td>
<td>10</td>
<td>25.7 ± 0.6</td>
<td>2.05 ± 0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dicaprylate 100 µg+4AP</td>
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Mean ± S.E.M.

a/b; c/d; e/f; e/g: P < 0.01.

Optical density is expressed as number of counts. Canalco microdensitometer (Model E) counts every 20 mm² of the area bounded by the tracing line and horizontal line.
Supporting this view, Bruce and Ramilrez (1970) found in rats that a clear inhibition of lactation was observed by intramammary estrogen implants, while direct intrapituitary estrogen was followed by a marked stimulation of lactation.

The pituitary graft is known to promote mammary growth in this strain of intact virgin mouse (Nagasawa et al., 1967b). The antagonistic action between estrogen and mammogenic hormones, especially prolactin, on mammary growth has demonstrated in the present results that isologous pituitary grafts induced conspicuous regeneration of the regressed mammary gland by hexestrol dicaprylate. The mammary regeneration by pituitary grafts would mainly be due to the direct stimulation of the gland by prolactin, since prolactin is the principal mammogenic hormone in this species (Nandi, 1959; Yanai and Nagasawa, 1971a). The vaginal smears in mice given both pituitary grafts and hexestrol dicaprylate showed the continued diestrus, indicating that progesterone, one mammogenic hormone, was secreted continuously from the ovary by the luteotropic action of prolactin. Therefore, the indirect effect of prolactin on mammary regeneration through ovary should also be taken into consideration. Similar results on the competitive effect of estrogen and prolactin have recently been obtained in carcinogen-induced mammary tumors of rats. The pituitary grafting (Nagasawa and Yanai, 1971) or prolactin injection (Meites et al., 1971) counteracted significantly the inhibition by estrogen of the incidence and growth of 7, 12-dimethylbenz(a)-anthracene-induced mammary tumors.

Acknowledgment

Our sincere thanks are due to Associate Prof. H. Mizuno, Shizuoka University, Shizuoka, for reading the manuscript and for his valuable comment. We also thank Mr. H. Ono, Takeda Chemical
Industries, Ltd., Tokyo, for his kind supply of hexestrol dicaprylate.

References