Effects of Neonatally Administered Sex Steroids on 7, 12-Dimethylbenz(a)anthracene-induced Mammary Neoplasia in Rats

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SUMMARY

Female Sprague-Dawley 5-day-old rats were given s.c. either 0.05 ml sesame oil, 1.25 mg progesterone, 0.1 mg estradiol benzoate, or 1.25 mg testosterone propionate. At 55 days of age all rats were given 20 mg of 7, 12-dimethylbenz(a)anthracene (DMBA) by stomach tube. All rats were killed at 190 days of age. Both the estradiol and the testosterone propionate treatments decreased the mammary adenocarcinoma response to DMBA and increased the mammary fibroadenoma response to DMBA. Both estradiol benzoate- and testosterone propionate-treated rats exhibited fewer developing ovarian follicles and no corpora lutea. Only testosterone propionate-treated rats exhibited lactation. Progesterone treatment increased the mammary adenocarcinoma response to DMBA and was without effect on the mammary fibroadenoma response to DMBA and ovarian structure.

INTRODUCTION

A single feeding of DMBA to young, adult, female Sprague-Dawley rats induces mammary cancer in almost all of the animals and benign mammary tumors in many of them (3). The ovaries and anterior pituitary are essential for development and growth of spontaneous or carcinogen-induced mammary tumors in rats under most conditions (10). The administration of large doses of sex hormones to neonatal female rats results in such profound disturbances in the development of the reproductive system that these rats do not exhibit estrus cycles after they reach maturity and they remain infertile (12). Since the neonatal administration of only testosterone propionate has been studied (5, 6, 15) on the subsequent development of mammary tumors in response to DMBA, a comparative study on the effects of testosterone propionate, estradiol benzoate, or progesterone was prosecuted.

MATERIALS AND METHODS

Pregnant rats were purchased from Sprague-Dawley, Inc., Madison, Wis. The female offspring were given 0.05 ml of sesame oil, 0.1 mg of estradiol benzoate, 1.25 mg of testosterone propionate, or 1.25 mg of progesterone in 0.05 ml of sesame oil s.c. on the 5th day of age. The female offspring of each mother were distributed to as many experimental and control groups as possible to prevent genetic bias. On the 55th day of age all of these rats were given 20 mg of DMBA in 4 ml of sesame oil by stomach tube. A similar experiment was done simultaneously, omitting the DMBA treatment. Ten rats from all 4 groups were killed on the 55th day of age, before DMBA administration. Mammary neoplasms were removed at autopsy and during the course of the experiment if the neoplasms became larger than 3 cm in the largest diameter. All mammary neoplasms were sectioned and stained and given a pathological classification following published criteria (14) of either adenocarcinoma or a combined classification of fibroadenoma-adenofibroma. At autopsy, body, ovarian, and uterine weights were obtained. Ovaries were sectioned and stained and the numbers of follicles and corpora lutea were recorded. Rats were maintained on commercial rat chow and water ad libitum in a temperature (72 ± 3°F) and humidity (55 ± 5%)-controlled room under conditions of 12-hr light per day.

Group means were tested using the t test and the incidence of rats with mammary neoplasms or the total number of mammary neoplasms per group was tested by 4-fold contingency tables (7) or by methods described previously (13).

RESULTS

The number of animals at 55 days of age, the neoplastic results, the organ and body weights, and measures of ovarian structure are given in Table 1. Survival from 55 through 190 days of age exceeded 95% in all groups. Survival from 2 to 55 days of age exceeded 90% in all groups. When rats were killed at 55 days of age, those that received either testosterone propionate or estradiol benzoate at 5 days of age had smaller ovarian weights and earlier vaginal openings, significant at the 5% level of confidence by t test, than those rats that received either oil or progesterone.
The number of maturing ovarian follicles was reduced in estradiol benzoate or testosterone propionate as compared hormones had no corpora lutea. Lactation, with milk being testosterone propionate, and animals treated with these 2 animals that received neonatal estradiol benzoate or were not changed by any of the steroids given neonatally. Broadenoma response to DMBA. Body and organ weights progesterone was without effect on the mammary fi 

at 5 days of age. Mammary neoplasms were found only in animals treated with DMBA. The number of rats with mammary neoplasms of both types in animals receiving DMBA was reduced only in the group that received neonatal estradiol benzoate. However, the number of animals with mammary adenocarcinomas and the total number of mammary adenocarcinomas were reduced in animals receiving DMBA by both estradiol benzoate and testosterone propionate treatment. Neonatal treatment with progesterone did not change the number of animals with mammary adenocarcinomas but the total number of mammary adenocarcinomas was higher in those animals that received progesterone and DMBA than in those animals that received oil plus DMBA. Neonatal treatment with testosterone propionate increased the number of animals with mammary adenofibromas. The total number of mammary adenofibromas found in this group were distributed almost equally between lactating and nonlactating rats.

DISCUSSION

Since DMBA has the capacity to induce 2 different types of mammary neoplasms, adenocarcinomas and fibroadenomas (3), it seems reasonable to discuss the mammary neoplastic response in terms of each type of tumor (13). Both estradiol benzoate and testosterone propionate depressed the mammary adenocarcinoma response to DMBA while at the same time both estradiol benzoate and testosterone propionate increased the mammary adenofibroma response to DMBA. Correlated with these effects was the finding that estradiol benzoate and testosterone propionate depressed ovarian function in that the number of ovarian follicles was reduced and no corpora lutea were found. It has been noted previously that neonatal estrogen treatment produces a lack of cyclic estrus and ovaries devoid of corpora lutea (12) and that the neonatal treatment with androgen produces a state of constant estrus and ovaries lacking corpora lutea (1). Thus, both neonatally administered androgen and estrogen produce an apparently similar depression of ovarian function. On the other hand, only neonatal treatment with androgen has been reported to modify mammary structure and function. Stern and Mickey (15), Dao (2), and Kovacs (5) have reported finding, as was found in the present experiment, lactating mammary tissue modify mammary structure and function. Stern and Mickey (15), Dao (2), and Kovacs (5) have reported finding, as was found in the present experiment, lactating mammary tissue

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Table 1

Mammary neoplasia, organ, and body weights in female Sprague-Dawley rats at 190 days of age

DMBA was given at 55 days of age, and sex steroids were given at 5 days of age. When number or mean is followed by Footnote c, the value is different from DMBA treatment at p = 0.05; Footnote d, p = 0.01 by either t test or $\chi^2$ test. Similarly, each hormonally treated group has been tested against hormone plus DMBA.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rats with</th>
<th>Total no. of mammary neoplasms</th>
<th>Body wt (g)</th>
<th>Organ wt (mg)</th>
<th>Ovarian structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil + DMBA</td>
<td>33</td>
<td>20</td>
<td>19</td>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td>Estradiol benzoate + DMBA</td>
<td>31</td>
<td>10*</td>
<td>4*</td>
<td>7</td>
<td>28</td>
</tr>
<tr>
<td>Testosterone propionate + DMBA</td>
<td>36</td>
<td>18</td>
<td>8*</td>
<td>12*</td>
<td>24</td>
</tr>
<tr>
<td>Progesterone + DMBA</td>
<td>30</td>
<td>23</td>
<td>19</td>
<td>7</td>
<td>53*</td>
</tr>
<tr>
<td>Oil</td>
<td>9</td>
<td>0*</td>
<td>0*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Estradiol benzoate</td>
<td>9</td>
<td>0*</td>
<td>0*</td>
<td>0</td>
<td>0*</td>
</tr>
<tr>
<td>Testosterone propionate</td>
<td>9</td>
<td>0*</td>
<td>0*</td>
<td>0*</td>
<td>0*</td>
</tr>
<tr>
<td>Progesterone</td>
<td>7</td>
<td>0*</td>
<td>0*</td>
<td>0*</td>
<td>0*</td>
</tr>
</tbody>
</table>

* AC, adenocarcinoma of mammary origin; AF, adenofibroma of mammary origin.
* Mean ± S.D.
treated with either neonatal estradiol benzoate or testosterone propionate.

The neonatal treatment with progesterone was without apparent effect on the mammary adenofibroma response to DMBA while, at the same time, progesterone tended to stimulate the number of mammary adenocarcinomas found after DMBA treatment. The observed lack of effect of neonatal progesterone treatment on ovarian structure, noted in the present experiment, is in keeping with some of the reports on neonatal treatment with progesterone and ovarian function (4, 8, 11).

There are only a few reports on the influences of neonatal sex hormone treatment and DMBA-induced mammary neoplasia and lactation and these pertain to testosterone propionate. Dao (2) found an 81% incidence of rats with mammary neoplasia 5 months after DMBA administration to female Sprague-Dawley rats that had been given 1 mg of testosterone propionate per day for the 1st 5 days of life. However, a lack of information as to the pathological type of mammary tumors makes it difficult to compare his report to the current finding of testosterone propionate-produced stimulation of the mammary fibroadenoma and inhibition of the mammary adenocarcinoma response to DMBA. Stern and Mickey (15) reported only that 11 or 23 female Sprague-Dawley rats developed mammary neoplasia following 1.25 mg of testosterone propionate given at 5 days of age and DMBA at 50 days of age and studied for 100 days after the carcinogen. Again, without data on the types of mammary tumors makes it difficult to compare his report to the current finding of testosterone propionate-produced stimulation of the mammary fibroadenoma and inhibition of the mammary adenocarcinoma response to DMBA.

The few animals that received either testosterone propionate or estradiol benzoate at 5 days of age and were killed at 55 days of age had smaller ovarian weights and relatively few ovarian follicles were found at the end of the experiment in those animals that received either neonatal testosterone propionate or estradiol benzoate and DMBA at 55 days of age. Thus the decreased adenocarcinoma response and the increased fibroadenoma responses to DMBA in these animals must be ascribed to the continuing effects of neonatal testosterone propionate or estradiol benzoate both before and after DMBA administration.

**REFERENCES**


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