The Lack of Effect of 7,12-Dimethylbenz(a)anthracene on the Endocrine Function of the Gonads

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Summary

7,12-Dimethylbenz(a)anthracene modified the response of the accessory reproductive organs of immature male and female rats to human chorionic gonadotropin. Animals receiving DMBA failed to gain weight, and it was shown that this failure impaired the response to the larger dose of HCG.

No abnormality could be detected histologically in the heavily luteinized ovaries of rats primed with pregnant mares' serum and HCG, then treated with DMBA. There was no difference in ascorbic acid content between these ovaries and those of control rats. It is concluded that DMBA has no direct action on the endocrine function of the gonads.

Introduction

Since the report by Yang et al. (11) that carcinogenic polynuclear aromatic hydrocarbons as a group bear steric resemblances to steroid hormones, there has been considerable interest in the effect of these compounds on the adrenals, gonads, and pituitary. Huggins and Morii (5) found that DMBA induced adrenal necrosis and hemorrhage when fed to adult rats. There have been conflicting reports on the effect of DMBA on the gonads.

Wong et al. (10) showed that DMBA, when fed to young adult female rats, was not only capable of inducing acute adrenal necrosis, but in half the animals also caused necrosis of the corpora lutea. On the other hand, Ford and Huggins (4) could find no abnormality in the ovaries of rats given a single i.v. injection of DMBA. They also noted that DMBA caused damage to the testis, but lesions were confined to cells that actively synthesize DNA, i.e., spermatogonia and resting spermatocytes.

Stern et al. (8) reported the initiation of vaginal cycling in androgen-sterile rats within 24-72 hr of DMBA administration. Since DMBA also augmented lactogenesis in these animals, the vaginal changes were thought to be secondary to prolactin release. A decrease in the weights of the pituitary gland, the ovaries, and the uterus of carcinogen-treated rats was also found.

The purpose of the present paper is to investigate the direct effect of DMBA on the gonads. Two methods have been employed. HCG has been used to stimulate steroidogenesis in rat ovaries and testes. Weight changes in the accessory reproductive organs resulting from this treatment have been compared in control and DMBA-treated animals. In addition, the action of the carcinogen on heavily luteinized ovaries, produced by administering large doses of HCG and PMS to rats, has been studied.

Materials and Methods

The experimental animals consisted of male and female Wistar rats from an inbred colony, aged from 20 to 22 days and weighing 30-40 gm.

DMBA was given by gastric tube to half the female animals in a dose of 10 mg/rat, each dose being dissolved in 1 ml of olive oil. The control group of animals received only the olive oil.

The DMBA-treated and control groups of animals were each subdivided into 3 groups containing at least 12 animals. HCG was administered to 2 of the groups in each series in a total dose of 1 IU and 2 IU/rat, respectively, while the 3rd group was given saline. After each dose of HCG was dissolved in 1.5 ml of saline, 0.5 ml was injected s.c. for 3 days.

Twenty-four hr after the last injection the animals were killed with ether and weighed. The uteri were dried after fixing overnight in Bouin's fluid and were then weighed on a torsion balance.

The male animals were treated in a similar manner, but the doses of HCG were increased to 2 IU and 4 IU/rat, respectively. The prostate and seminal vesicles were weighed, and the testes were examined histologically.

In a further experiment, 2 groups of immature female rats were primed with HCG and PMS according to the schedule of Sakiz and Guillemin (7). Five days after the final injection of gonadotropin, each rat in 1 group was given 20 mg of DMBA and 1 ml of olive oil, while the control group was fed only olive oil. Three days later the animals were sacrificed, and a small portion of each ovary was examined microscopically with the use of hematoxylin and eosin staining. The remainder of each ovary was homogenized in 2.5 ml of 2.5% metaphosphoric acid, and the ascorbic acid content of each ovary was determined by the method of Dekanski and Harvie (3). The results were expressed as mg of ascorbic acid/100 gm of ovary.

Results

Table 1 shows the uterine, prostatic, and seminal vesicle weights after stimulation with HCG in the DMBA and control groups of rats. Although there is no significant difference between the 2 groups in the effect of 1 IU HCG, there was a difference in the response to 2 IU. The larger dose produced a marked rise in uterine weight in the control group, but the weight in the
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**TABLE 1**

| Effect of HCG on Weight of Uterus, Prostate, and Seminal Vesicles in DMBA-treated Rats and Controls |
|---|---|---|
| Mean uterine wt (mg ± S.E.) | Mean prostate wt (mg ± S.E.) | Mean seminal vesicle wt (mg ± S.E.) |
| DMBA (44 rats) | Control (45 rats) | DMBA (44 rats) | Control (44 rats) | DMBA (44 rats) | Control (44 rats) |
| Saline | 18.1 ± 0.43 | 19.2 ± 12.2 | 28.9 ± 2.3 | 28.8 ± 1.1 | 10.3 ± 0.66 | 13.5 ± 1.6 |
| 1 IU HCG | 89.1 ± 7.75 | 78.4 ± 12.2 | 42.6 ± 2.51 | 43.0 ± 2.29 | 16.0 ± 1.51 | 17.0 ± 1.64 |
| 2 IU HCG | 94.4 ± 10.5 | 141.3 ± 6.35 | (125.0 ± 5.5)* | (137.0 ± 5.5) | 43.1 ± 2.62 | 55.8 ± 3.1 |
| 4 IU HCG | 43.1 ± 2.62 | 55.8 ± 3.1 | (50.0 ± 6.0) | (59.0 ± 2.1) | 16.0 ± 1.4 | 22.0 ± 1.63 |

* The figures in parentheses are the results obtained when DMBA was fed to animals that were an average of 10 gm heavier than the controls. At the completion of the experiment there was no significant difference between the mean body weights of the 2 groups.

**TABLE 2**

| Ovarian Weight and Ascorbic Acid Content in DMBA-treated and Control Rats Primed with HCG and PMS |
|---|---|
| Group | Ovarian wt. | Ascorbic acid (mg/100 gm ovary ± S.E.) |
| Control | 119 ± 8.4 | 54.2 ± 2.7 |
| DMBA | 123 ± 8.0 | 47.8 ± 4.3 |

DMBA-treated animals was no greater than that produced by 1 IU.

When the prostatic and seminal vesicle weights were used as indices of HCG activity, the DMBA-treated group again showed a very much poorer response to the larger dose of HCG.

DMBA in a dose of 10 mg had no effect on the histology of the testis. On the other hand, when a dose of 20 mg/rat was used there was a high mortality rate, but in the survivors there was a marked reduction in both spermatogonia and spermatocytes. The interstitial cells were normal.

There was no difference in the histologic appearance of the ovaries from DMBA-treated and control rats primed with HCG and PMS. The ovarian weights and ascorbic acid contents were similar in the 2 groups (Table 2). No evidence of adrenal necrosis was found.

**Discussion**

Following the discovery by Huggins and Morii (5) that DMBA caused adrenal cortical necrosis, it was shown that this effect was related to corticosterone synthesis (1, 2, 6, 9). The present investigation studied the action of DMBA on the gonads, since these organs are also concerned in steroidogenesis.

From the results reported here it is concluded that DMBA has no effect on the endocrine function of the gonads. There was no difference between the DMBA-treated and control rats in the weights of the unstimulated accessory reproductive organs and in the weights, histologic appearances and ascorbic acid contents of ovaries from rats primed with HCG and PMS. DMBA had no effect histologically on the Leydig cells.

A difference between the 2 groups was found in the response of the accessory reproductive organs to HCG. Two interpretations of this result are possible. (a) The DMBA may cause a partial block of ovarian steroid synthesis so that although these organs respond satisfactorily to a small dose of HCG, they are not capable of being more strongly stimulated. (b) A more likely explanation of the findings was obtained by a study of the weights of the animals at the beginning and the end of the experiment. Although initially the mean weight of all the animals was 35 gm, on the 4th day the control animals weighed 45 gm while the DMBA-treated animals had failed to gain weight, the difference between the 2 groups being significant (P = 0.001). It was possible, therefore, that it was the size of the animal rather than the DMBA that limited the response of the uterus and other organs to the larger doses of HCG.

In order to test this possibility, therefore, a further series of experiments was performed in which the control animals were injected exactly as before, but the DMBA was fed to animals that were an average of 10 gm heavier. At the end of the experiment there was no significant difference between the mean weights of the DMBA-treated rats and the controls. In contrast to the previous experiments there was closer agreement in the responses to HCG in the 2 groups (Table 1). Therefore, the effect of DMBA in this experiment was nonspecific; the gonad was not directly affected.

Ford and Huggins (4) reported that a single i.v. injection of DMBA into adolescent rats caused a selective destruction of the spermatogonia and resting spermatocytes. Although no such damage occurred in the present series with a dose of 10 mg of DMBA given orally, the lesions were produced with a dose of 20 mg. Steroidogenesis in the testis takes place in the Leydig cells and perhaps also in the Sertoli cells. The destruction of the spermatogonia and spermatocytes by DMBA cannot therefore depend on steroid synthesis.

Dao and Tanaka (2) frequently observed necrosis of corpora lutea in DMBA-treated rats but believed this represented their normal involution. On the other hand Wong et al. (10) noted the same lesions in 50% of their experimental animals and thought the involuntory changes were exaggerated. Furthermore, they postulated that the necrosis of corpora lutea together with adrenal necrosis would deplete the main sources of steroids in the body and would result in hormonal imbalance, which may enhance the effect of the carcinogen. No evidence to support this hypothesis was found in the present investigation.

It appears from the work of Stern et al. (8) that DMBA has the opposite effect on the ovary—namely, stimulation secondary...
to prolactin release from the pituitary. The decrease in the weights of the uterus, ovaries, and pituitary that they reported in carcinogen-treated rats could be attributed to the generalized toxic effect of DMBA found in the present series.

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References

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