Inhibitory Effect of Polynuclear Hydrocarbons and Amphenone Analogs on Induction of Acute Adrenal Necrosis by 7,12-Dimethylbenz[a]anthracene*

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SUMMARY

A single feeding of an effective dose of any of the six polycyclic aromatic hydrocarbons including four carcinogens—DMBA, 3-MC, BP, and BA—and two noncarcinogens—anthracene and phenanthrene—48 hours prior to the administration of 30 mg. of DMBA completely inhibited the DMBA-induced adrenal necrosis. The minimal effective dose (to induce a 100 per cent protection against the DMBA-induced adrenal necrosis) for the four carcinogenic polycyclic hydrocarbons was 10 mg., and that for two noncarcinogens was 25 mg. for anthracene and 100 mg. for phenanthrene. Among the four carcinogens, 3-MC appears to provide the most effective protection against DMBA-induced adrenal necrosis. It is most remarkable to observe that a non-necrotizing dose of DMBA inhibited the necrosis-inducing effect of the same compound. The experimental results indicate that the presence of a critical amount of adrenocortical steroids in the adrenal gland is a prerequisite to the induction of necrosis by DMBA.

The present investigation demonstrated that, whereas Metopirone was partially effective in inhibition of DMBA-induced adrenal necrosis, o,p'-DDD, also an amphenone analog, was totally ineffective.

Recently Currie et al. reported that the administration of 2-methyl-1, 2-bis(3-pyridyl)-1-propynone (Metopirone, or Ciba SU 4885) prior to the feeding of 7,12-dimethylbenz[a]anthracene (DMBA) inhibited the induction of acute massive necrosis by the carcinogen (3). Whatever the protective mechanism of Metopirone, it is known to interfere with the synthesis of cortisol and corticosterone (2). This consideration led to our experiments with 3-methylcholanthrene (3-MC), which was shown to inhibit corticosterone synthesis in the adrenal gland in rats (4). We discovered that administration of two doses of 30 mg. of 3-MC prior to the feeding of DMBA completely inhibited the necrosis-inducing effect of DMBA (5). Earlier, Morii and Huggins reported that the adrenal cortex of immature rats was refractory to DMBA but became susceptible if ACTH was given (8). These authors showed that the corticosterone content of the adrenal gland in young rats was lower than in adult rats. On the basis of these various observations, it became evident that the level of corticosterone in the adrenal glands is critical for the induction of cortical necrosis by DMBA.

The remarkable phenomenon of 3-MC inhibition of the necrosis-producing effect of DMBA raises some questions: (a) Is this effect unique to 3-MC, or is it shared by other polycyclic hydrocarbons? (b) Is the inhibitory effect of a polycyclic hydrocarbon on induction of adrenal necrosis related to the chemical structure of the hydrocarbon? (c) Does inhibition of adrenal necrosis by 3-MC (and possibly by other hydrocarbons) have any effect on the induction of cancer in the mammary gland in rats?

This paper reports our experiments on the inhibitory effects of a series of six polycyclic hydrocarbons, four carcinogens, and two noncarcinogens on the induction of adrenal necrosis by DMBA. In addition, a comparison was made of the effects of two known adrenal inhibitors, Metopirone and 1, 1-dichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl)ethylene (o,p'-DDD), on the induction of
adrenal necrosis. In these studies, we have found that a single effective dose of any of six polycyclic hydrocarbons, when fed to the rats 48 hours prior to the administration of DMBA, completely inhibited the necrosis-producing effect of DMBA. Most remarkable was the observation that a single feeding of a non-necrotizing dose of DMBA 48 hours prior to the administration of a necrotizing dose of DMBA also inhibited adrenal necrosis completely. Among the amphenone analogs, it was found that Metopirone partially inhibited the induction of acute necrosis by DMBA, but o,p'-DDD was ineffective at the dose used in the present experiments.

MATERIALS AND METHODS

Sprague-Dawley female rats, 55–60 days old and weighing 165–189 gm., were used in all experiments, and there were ten rats in each group, unless stated otherwise.

The polycyclic hydrocarbons used in this study were: 7, 12-dimethylbenz[a]anthracene (DMBA), 3-methylcholanthrene (3-MC), benzo[a]pyrene (BP), benz[a]anthracene (BA), phenanthrene, and anthracene. All carcinogens were dissolved in olive oil. Concentrations in mg/ml were as follows: 30, 10, 3, and 1 for 3-MC; 30, 10, 5, and 1 for DMBA; 30, 10, 3, and 1 for BP; 50, 25, 10, and 5 for benz[a]anthracene; 100, 50, 25, and 10 for phenanthrene; and 50, 25, 10, and 5 for anthracene.

Metopirone ditartrate was dissolved in olive oil in a concentration of 10 mg/0.1 ml and was given intraperitoneally. o,p'-DDD was dissolved in olive oil in a concentration of 16 mg/0.1 ml, and also was injected intraperitoneally.

In experiments concerned with the effects of various polycyclic hydrocarbons on the induction of adrenal necrosis by DMBA, the basic design was uniform. With the exception of DMBA, which was given in three different doses to three different groups of rats, all of the other compounds were given in four different doses to four different groups of rats by a single feeding and then a single feeding of 30 mg of DMBA 48 hours later. All rats were killed 3 days after the feeding of 30 mg of DMBA. Thus, there were 923 groups of rats, all of the other compounds were given in four different doses to four different groups of rats: (a) Metopirone, 10 mg/0.1 ml intraperitoneally every 4 hours for three doses and a single feeding of 30 mg of DMBA 4 hours later; (b) o,p'-DDD, 16 mg/0.1 ml intraperitoneally for two daily doses and then a single feeding of 30 mg of DMBA 24 hours later; (c) o,p'-DDD, 16 mg/0.1 ml intraperitoneally every 4 hours for two doses, and then a single feeding of 30 mg of DMBA 24 hours after the last dose; (d) o,p'-DDD, 16 mg/0.1 ml intraperitoneally every 4 hours for three doses and then a single feeding of 30 mg of DMBA 4 hours later; and (e) o,p'-DDD, 16 mg/0.1 ml intraperitoneally every 4 hours for three doses and then a single feeding of 30 mg of DMBA 4 hours later. All rats were killed 3 days after the feeding of 30 mg of DMBA.

As controls for this entire study, there were five groups of rats: (a) intact rats receiving no treatment, (b) rats receiving a single feeding of 30 mg of DMBA and killed 3 days later, (c) rats receiving 10 mg of Metopirone every 4 hours for three doses, (d) rats receiving 16 mg of o,p'-DDD daily for two doses, and (e) rats receiving 16 mg of o,p'-DDD every 4 hours for three doses. All rats were killed 3 days later.

At the time of autopsy, all endocrine glands were trimmed and weighed. Adrenals were examined carefully under good illumination. All adrenals and ovaries were fixed in formalin, and sections were stained with hematoxylin and eosin.

RESULTS

Effect of polycyclic hydrocarbons on the induction of adrenal necrosis by DMBA.—When 30 mg of DMBA was given to rats, 100 per cent of the animals developed acute massive necrosis and hemorrhage, and five of ten rats died on the 3rd day shortly before the scheduled time of autopsy. The mean adrenal weight in ten intact rats was 28.2 ± 2.5 mg/100 gm body weight. The mean adrenal weights in rats receiving 30 mg of DMBA was markedly increased, mainly because of massive hemorrhage and necrosis, which were severest on the 3rd day after the carcinogen feeding.

Although necrosis of corpora lutea of the DMBA-treated rats was observed frequently, it was believed that most of them represented normal involutionary process. Using the criteria of Wong et al. (10) we observed diffused necrosis of lutein cells with leukocytic infiltration in only one of the ten rats receiving 30 mg of DMBA. In the remaining nine rats, however, the necrosis was patchy, resembling the involutionary necrosis of corpora lutea in the normal rats.
The results summarized in Table 1 show that all the polycyclic hydrocarbons are effective in protecting the adrenal cortex against DMBA-induced adrenal necrosis. It is most remarkable that 10 mg. of DMBA given prior to the feeding of 30 mg. of DMBA completely inhibited the necrosis-producing effect of the same hydrocarbon. Pretreatment with 1 mg. of DMBA, however, failed to provide the same protective effect as 10 mg. of the compound.

The minimal effective doses of other polycyclic hydrocarbons to inhibit the induction of adrenal necrosis by DMBA are: 10 mg. for S-MC; 10 mg. for BP; 10 mg for benz[a]anthracene; 25 mg. for anthracene; and 100 mg. for phenanthrene. It appears that among these six polycyclic hydrocarbons S-MC is the most effective compound in the inhibition of induction of adrenal necrosis by DMBA. Adrenal necrosis was prevented in 70 per cent of the rats pretreated with as little as 1 mg. of S-MC, whereas necrosis occurred in 90–100 per cent of the rats pretreated with the same doses of DMBA, BP, and benz[a]anthracene. The two noncarcinogenic hydrocarbons, anthracene and phenanthrene, are significantly less effective inhibitors of adrenal necrosis than the carcinogenic hydrocarbons.

The gross appearance of the adrenal glands in rats receiving 30 mg. of DMBA was striking. They were dark red in color, greatly enlarged and engorged with blood. In rats receiving a single effective dose of any of the six polycyclic hydrocarbons prior to DMBA administration, the adrenals were bright yellow in color with no evidence of any hemorrhage or necrosis.

Microscopically, massive hemorrhage and extensive karyolysis throughout the zona fasciculata and zona reticularis are the characteristic pictures on day 3 in rats receiving a single oral dose of 30 mg. of DMBA. In contrast, the microscopic appearance of the adrenal cortex in rats pretreated with any of the six polycyclic aromatic hydrocarbons were normal, with no evidence of necrosis or degenerative changes (Figs. 1 and 2).

**Effect of amphenone analogs on the induction of adrenal necrosis by DMBA.**—The results are summarized in Table 2. No hemorrhage or necrosis has been observed grossly in adrenal glands in rats treated with Metopirone prior to DMBA administration, the adrenals were bright yellow in color with no evidence of any hemorrhage or necrosis.

TABLE 2

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean adrenal weights (mg/100 gm body wt)</th>
<th>Adrenal necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no treatment)</td>
<td>28.6 ± 2.5</td>
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</tr>
<tr>
<td>DMBA (30 mg.)</td>
<td>48.5 ± 7.8</td>
<td>10/10</td>
</tr>
<tr>
<td>DMBA (10 mg.)</td>
<td>28.8 ± 1.9</td>
<td>0/10</td>
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<tr>
<td>DMBA (5 mg.)</td>
<td>30.3 ± 4.1</td>
<td>2/10</td>
</tr>
<tr>
<td>DMBA (1 mg.)</td>
<td>49.3 ± 8.3</td>
<td>10/10</td>
</tr>
<tr>
<td>S-MC (10 mg.)</td>
<td>29.1 ± 2.1</td>
<td>0/10</td>
</tr>
<tr>
<td>S-MC (5 mg.)</td>
<td>29.3 ± 3.7</td>
<td>1/10</td>
</tr>
<tr>
<td>S-MC (1 mg.)</td>
<td>34.5 ± 12.3</td>
<td>5/10</td>
</tr>
<tr>
<td>BP (10 mg.)</td>
<td>30.7 ± 3.8</td>
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<tr>
<td>BP (5 mg.)</td>
<td>37.2 ± 13.7</td>
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<tr>
<td>BP (1 mg.)</td>
<td>48.3 ± 11.7</td>
<td>9/10</td>
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<tr>
<td>Benz[a]anthracene (10 mg.)</td>
<td>28.8 ± 2.4</td>
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<tr>
<td>Benz[a]anthracene (5 mg.)</td>
<td>32.1 ± 4.8</td>
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<td>49.6 ± 7.6</td>
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<tr>
<td>Anthracene (25 mg.)</td>
<td>31.3 ± 5.4</td>
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<td>Anthracene (10 mg.)</td>
<td>43.9 ± 17.0</td>
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<tr>
<td>Anthracene (5 mg.)</td>
<td>50.5 ± 12.3</td>
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<tr>
<td>Phenanthrene (100 mg.)</td>
<td>30.2 ± 1.4</td>
<td>0/10</td>
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<td>Phenanthrene (50 mg.)</td>
<td>35.4 ± 8.3</td>
<td>2/10</td>
</tr>
<tr>
<td>Phenanthrene (25 mg.)</td>
<td>40 ± 13.9</td>
<td>8/10</td>
</tr>
</tbody>
</table>

* A single feeding of 30 mg. of DMBA was given 48 hours after pretreatment with one of the six hydrocarbons in all the experiments.

† No. animals with adrenal necrosis/no. animals.

± Standard deviation.
dying on the day of autopsy. It should be noted that the rats pretreated with 3-MC prior to DMBA were massive necrotic and hemorrhagic at the time of autopsy. The mean adrenal weight in rats receiving 10 mg. of DMBA or 30 mg. of 3-MC totally nullifies the protective effect of these hydrocarbons against the necrosis-inducing effect of DMBA. It should be mentioned that the adrenal weights in rats receiving only o,p'-DDD were uniformly larger than those of the control rats without any treatment. Hertz (6) reported enlargement of the adrenal glands and accumulation of fat in the cortical cells of the inner two zones of the adrenal cortex in amphenone-treated rats.

Effect of a prolonged interval between pretreatment with polycyclic hydrocarbons and feeding of DMBA on adrenal necrosis.—Administration of DMBA 7 days following pretreatment of the rats with 10 mg. of DMBA or 30 mg. of 3-MC totally nullifies the protective effect of these hydrocarbons against the necrosis-inducing effect of DMBA.

The mean adrenal weights were $47.3 \pm 7.5$ mg/100 gm body weight in five rats receiving 10 mg. of DMBA as pretreatment 7 days prior to the administration of 30 mg. of DMBA, and three rats in this group were dying on the day of autopsy. In the group in which the rats receiving 30 mg. of 3-MC daily for two doses and then 7 days later 30 mg. of DMBA, the adrenal glands in all five rats were massively necrotic and hemorrhagic at the time of autopsy. The mean adrenal weight in this group of rats were $62 \pm 2.8$ mg/100 gm body weight and four of five rats in this group were dying on the day of autopsy. It should be noted that in this experiment the adrenal weights in rats pretreated with 3-MC prior to DMBA were significantly ($P < 0.01$) higher than those in rats receiving 30 mg. of DMBA only.

DISCUSSION

The experimental results presented in this paper showed that inhibition of DMBA-induced adrenal necrosis was not unique to 3-MC. Other polycyclic aromatic hydrocarbons, including benz[a]pyrene, benz[a]anthracene, phenanthrene, and anthracene were equally as effective as 3-MC in the inhibition of induction of adrenal necrosis by a large dose of DMBA. Even a non-necrotizing dose of DMBA given prior to the administration of a necrotizing dose of the same hydrocarbon effectively protects the adrenal cortex against necrosis.

Although the mechanism by which the polycyclic aromatic hydrocarbons inhibit DMBA-induced adrenal necrosis is not understood, the earlier observation of Morii and Huggins (8) and by us (5) suggested that the concentration of corticosterone in the adrenal gland was critical for the induction of adrenal necrosis. The fact that the polycyclic aromatic hydrocarbons can inhibit adrenal corticosterone synthesis (4), as does Metopirone, led us to believe that the mechanism by which the polycyclic hydrocarbons inhibit adrenal necrosis is similar to that by which Metopirone inhibits it. Since Metopirone is known to interfere with the synthesis of cortisol and corticosterone by inhibition of 11β-hydroxylation (2), it is suggested that polycyclic hydrocarbons are inhibitors of adrenal enzymes concerned with corticosteroid synthesis.

The compounds studied in the present investigation are all polycyclic aromatic hydrocarbons. If these compounds are inhibitors of enzyme systems concerned with the biosynthesis of corticosteroid hormones, it would appear that they impart their inhibitory effect at different levels along the biosynthetic pathways of corticosteroids in rats, since our earlier observation showed that, whereas 3-MC caused depletion of corticosterone level in the adrenal, a non-necrotizing dose of 10 mg. of DMBA did not produce the same effect (4). Experiments to elucidate the effects of the polycyclic aromatic hydrocarbons on biosynthesis of corticosteroid hormones in rat adrenals are under way in our laboratory.

That the inhibitory effect of these polycyclic hydrocarbons on induction of adrenal necrosis by DMBA is only temporary is clearly demonstrated by the experiments that delayed administration of DMBA after pretreatment with 3-MC nullifies the inhibition of DMBA-induced adrenal necrosis by 3-MC. In an earlier paper (4), we reported that following a maximal decline of the adrenal corticosterone level on the 8d and 4th day after feeding of 3-MC, it rose to the pretreatment level between the 5th and 8th day. The present observation of...
The transient inhibitory effect of 3-MC seems to provide additional evidence to the suggestion that the polycyclic hydrocarbons inhibit adrenal enzymes concerned with corticosteroid synthesis.

The lack of inhibition of DMBA-induced adrenal necrosis when rats were pretreated with o,p′-DDD is interesting but cannot be readily explained. o,p′-DDD is known to induce adrenal necrosis in dogs and consequently a decrease in adrenocortical hormone synthesis (9). It was not known, however, whether a non-necrotizing dose of this compound had the same effect. In our experiments it would appear that o,p′-DDD was unable to suppress rat corticosteroid synthesis, since it was not effective in inhibiting the DMBA-induced adrenal necrosis. It should be pointed out that, in the previous experiments in dogs, o,p′-DDD was administered orally. In the present experiments, o,p′-DDD was given intraperitoneally. It is doubtful, however, whether the different routes of administration have an influence upon the effect of o,p′-DDD.

The results of this study also demonstrate that there is a minimal effective dose for each hydrocarbon. It is noteworthy that the minimal doses of these hydrocarbons required to cause effective inhibition of adrenal necrosis seem to parallel the carcinogenic potency of these compounds. The effective dose of phenanthrene is almost 30 times that of the 3-MC. It should, however, be pointed out that 3-MC, not DMBA, appears to be the most effective compound in inhibiting the induction of adrenal necrosis by DMBA.

The data from the present investigation show a similarity to the findings in the study of the effect of the hydrocarbons on the suppression of sebaceous glands (1). In these experiments, it was shown that the degree of destruction of sebaceous glands by a group of aromatic polycyclic hydrocarbons was parallel to the carcinogenic potency of these compounds.

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REFERENCES
FIG. 3.—Necrosis affecting only a few cells here and there in the adrenal cortex of a rat receiving Metopirone 4 hours prior to the feeding of 80 mg. of DMBA. ×450.
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