On the Mechanism of Inducing Protection of the Adrenal Cortex against Injury from 7,12-Dimethylbenz(a)anthracene

I. Effects of Inducers on Benzpyrene Hydroxylase Activity

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Summary

Benzpyrene (BP) hydroxylase activity in the liver, small intestine, adrenal, and mammary gland of normal rats has been quantitatively assayed. There was only a very small amount of enzyme activity in the mammary gland. Administration of 3-methylcholanthrene (3-MC) induced a marked increase in BP hydroxylase activity in the liver and intestine, but was without effect on the enzyme level in the mammary gland and the adrenals. Mammary gland and adrenals are not sites for hydroxylation of polycyclic aromatic hydrocarbons.

The capacities of polycyclic aromatic “protectors” and inactive compounds to induce BP hydroxylase synthesis were compared. When the doses of inactive “inducers” were increased several-fold, the level of BP hydroxylase activity in liver and intestine changed only slightly but a marked increase of adrenal protective activity was observed.

Sequential administration of hydrocarbons induced considerably larger amounts of BP hydroxylase than were evoked by either hydrocarbon separately; the induction of enzyme was more than additive if the combinations were 3-MC + 7,12-dimethylbenz(a)anthracene (DMBA) and DMBA + DMBA but was less than an arithmetic sum if the combinations were 3-MC + 7,12-dimethylbenz(a)anthracene (DMBA) and DMBA + DMBA but was less than an arithmetic sum if the combinations were 3-MC + 3-MC and DMBA + 3-MC.

Actinomycin D, puromycin, and dl-ethionine inhibited 3-MC-induced stimulatory effect of BP hydroxylase activity. Under appropriate conditions of time, puromycin and dl-ethionine abolished the adrenal protection induced by aromatic hydrocarbons. Actinomycin D, under identical experimental conditions, had no effect on the adrenal protective activity induced by aromatic hydrocarbons. The possible mechanism by which protection of the adrenal cortex against injury from DMBA is discussed.

Introduction

After a single p.o. dose of 20 mg of DMBA2 in rats, 2 important events occur: mammary cancer is invariably induced, and the adrenal cortex is severely damaged (5, 18). When larger doses of DMBA are used, fatal adrenal crisis develops. It has also been discovered that the adrenal cortex can be protected from necrosis and hemorrhage by pretreatment of the rat with any of a considerable number of aromatic hydrocarbons (4, 6, 7). The phenomenon of protection is especially interesting in view of the fact that DMBA, although it brings about adrenal hemorrhage and necrosis, is itself also an effective protector.

The mechanism by which polycyclic aromatic hydrocarbons inhibit DMBA-induced adrenal damage is not understood. Many chemical substances foreign to the organism induce the synthesis of enzymes that metabolize these compounds. Among the foreign compounds that induce the synthesis of metabolizing enzymes are polycyclic aromatic hydrocarbons, such as 3-MC and BP. Administration of a small dose of 3-MC or BP to rats induces a marked increase of hydroxylating enzymes in the liver (1, 3). It is conceivable that a markedly increased concentration of hydroxylating enzymes in the liver and other sites following pretreatment with polynuclear hydrocarbons makes possible the metabolic inactivation of DMBA before it can reach the adrenal cortex in sufficient quantity to inflict damage on the gland.

In an earlier paper we reported the quantitative determination of BP hydroxylase in the liver and adrenals in rats pretreated with polycyclic aromatic hydrocarbons (10). The results suggested that the adrenals were not important sites for the hydroxylation of aromatic hydrocarbons.

In the present investigation the hydroxylating enzymes in the liver, adrenals, intestines, and mammary glands of rats receiving any of a number of polycyclic aromatic hydrocarbons were studied. The relationship between the inhibition of the stimulatory effect of 3-MC on BP hydroxylase activity by ethionine, actinomycin D, and puromycin and the induction of adrenal necrosis was also studied in order to determine whether the induction of synthesis of the enzymes is related to their protective activity.

Materials and Method

Female Sprague-Dawley rats, 60-65 days old and weighing 170-190 gm, were used in all experiments, and they were fed a commercial diet (Rockland diet) and were given water ad libitum. Each experimental group consisted of 5 rats, and was paralleled by a control group of 5 untreated rats.

The aromatic hydrocarbons to be studied were dissolved in sesame oil, and 1 ml of the solution of a compound was fed to each rat p.o. The rats were killed by decapitation 24 hr after the administration of the compound.
feeding of the hydrocarbon, and a piece of liver (approximately 20–30 mg), the adrenal glands, a segment of small intestine, and a pair of abdominoungual mammary glands were removed. The segment of intestine was opened, and the mucosa was scraped from the intestinal wall with a sharp scalpel blade. Homogenates of these materials were prepared in cold (0°–2°C) isotonic KCl solution in a Potter-Elvehjem homogenizer. The concentrations of the tissue homogenates were as follows: liver, 0.25%; adrenals, 1%; intestinal mucosa, 2.5%; and mammary gland, 2.5%. All tissues were homogenized and assayed individually.

BP hydroxylase activity was measured as described previously (8), which was a slight modification of the method of Wattenberg et al. (21). In our assays, instead of using NADPH we used a NADPH-generating system containing 0.05 ml of 10.4 mg/ml NADP, 0.002 ml of 140 KU/ml glucose-6-phosphate dehydrogenase, and 0.1 ml of 11.4 mg/ml glucose-6-phosphate. The fluorescence of the aqueous extract was determined in a Farrand fluorometer, Model A, equipped with a primary filter that transmits light maximally at 390 µm and a secondary interference filter with a peak wave length of 325 µm and a half-band width of 14 µm. Quinine sulfate solution (0.4 µg/ml in 1 N H₂SO₄) was used as a fluorescent standard. Fluorescence was standardized with 8-hydroxybenzpyrene in most of the experiments. Zero-time control values for the tissue and incubation mixture were subtracted from the values for the incubated flasks. A unit of enzyme is defined as the amount of enzyme producing a fluorescence change at 522 µm equivalent to the production of 1 µg of 8-hydroxybenzpyrene/min. It should be noted, however, that some of the experiments described in this paper were done before 8-hydroxybenzpyrene became available to us as a standard. In these experiments, enzyme activity was calculated as fluorescence/mg of tissue.

Influence of Ethionine on Protection of the Adrenal Cortex

There were 7 groups of 10 rats each in this experiment. Rats in 3 experimental groups were given 25 mg of dl-ethionine i.p. 8, 4, or 0.5 hr before a single feeding of a protective dose of 3 mg of 3-MC. In another 3 groups, dl-ethionine was given 8, 4, or 0.5 hr after a single feeding of 3 mg of 3-MC. Rats in 1 group were given 3-MC only, without ethionine, as controls. Twenty-four hr after the feeding of 3-MC, a single dose of 30 mg of DMBA was given to rats in all 7 groups. Autopsy was performed 72 hr after the feeding of DMBA. Adrenal glands were examined under good illumination for gross evidence of hemorrhage, and sections were prepared for histologic studies.

Effect of Actinomycin D and Puromycin on Protection of the Adrenal Cortex

There were 5 groups of rats in this experiment. In 2 groups, 20 µg of actinomycin D were given i.p. in 3 consecutive doses 2 hr before 3-MC, simultaneously with the feeding of 3 mg of 3-MC, and 2 hr after 3-MC. A single dose of 30 mg of DMBA was fed 6 hr after 3-MC in 1 group and 24 hr after 3-MC in the other group. In 2 other groups, 3 mg of puromycin were given in 3 consecutive doses exactly paralleling those of actinomycin D in the 1st 2 groups. In the 5th group, 3-MC and DMBA were given as in the other 4 groups, but without either actinomycin D or puromycin. All rats were killed 72 hr after DMBA feeding.

Results

Induction of BP Hydroxylase Synthesis in Tissues of Rats Treated with Aromatic Hydrocarbons

Chart 1 shows a marked increase in BP hydroxylase activity in liver from rats treated with either 3-MC or DMBA. The stimulation induced by 3-MC is far greater than that induced by DMBA. BP hydroxylase activity is also stimulated in the intestines of rats treated with aromatic hydrocarbons, but the increase is significantly less than that in the liver. That the degree of stimulation of enzyme synthesis by aromatic hydrocarbons is dose-dependent is clear.

The effect of aromatic hydrocarbons on BP hydroxylase in the adrenals is just the opposite of their effect in the liver and intestines. Instead of a stimulating effect, the hydrocarbons induced a decrease in enzyme synthesis in the adrenals. These results are consistent with the data reported by us earlier (10).

Experiments showed that normal mammary glands from either female or male rats (170–190 gm) contain very small amounts of the hydroxylating enzyme. There was no stimulation of enzyme synthesis in mammary glands after the administration of either 3-MC or DMBA.

The capacities of aromatic protectors and inactive compounds for induction of BP hydroxylase synthesis were compared. The results in Table 1 show that fluorene is an inactive BP hydroxylase inducer even at a dose level of 100 mg, but it effectively protects the adrenal cortex against injury by DMBA. Similarly,

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*Throughout this paper the term "protector" means any polycyclic aromatic hydrocarbon that, when given prior to the administration of 30 mg of DMBA, protects the adrenal cortex against injury by DMBA.

*Throughout this paper the term "inducer" denotes any polycyclic aromatic hydrocarbon that induces BP hydroxylase synthesis in the liver.
aromatic hydrocarbons that induce the synthesis of metabolizing enzymes in the liver and many other sites can result in the metabolic inactivation of subsequent doses of DMBA, experiments with sequential administration of protective aromatic hydrocarbons and DMBA were carried out. In these experiments, rats received a single feeding of a protective hydrocarbon, followed by a single dose of 30 mg of DMBA 24 hr later. The rats were killed 24 hr after the feeding of DMBA, and the liver, intestines, and adrenals were removed for enzyme assay. Each group contained 5 rats. For comparison, BP hydroxylase was also assayed in tissues from control groups in which the rats received only a single aromatic hydrocarbon.

The results illustrated in Chart 2 show that there was a greater increase in BP hydroxylase in the liver in rats receiving 2 hydrocarbons in sequential administration than in rats receiving a single hydrocarbon. Rats that received 3-MC + DMBA or DMBA + DMBA had an increase in BP hydroxylase far above that obtained in the liver in rats that received only 3-MC or DMBA. The increase resulting from these 2 sequential feedings of a protector and a challenger (DMBA) actually was an additive sum of the 2 single effects. The data for BP hydroxylase in the intestine were similar. The results suggest that an earlier protective dose of either 3-MC or DMBA did not cause the challenger DMBA to be inactivated metabolically so as its capacity to induce BP hydroxylase synthesis in the liver and intestines was concerned. It should be noted, however, that the values for the combinations of 2 hydrocarbons were less than additive if 10 mg of 3-MC instead of 30 mg of DMBA were given 2nd, in the sequence.

Influence of Ethionine on 3-MC-induced Increases in BP Hydroxylase and Protection of the Adrenal Cortex

There were 6 groups of 5 rats each in experiments concerning 3-MC-induced synthesis of BP hydroxylase and the influence of ethionine on the process. A dose of 3 mg of 3-MC was administered p.o., and 25 mg of ethionine were given i.p. 8, 4, or 0.5 hr either before or after the feeding of 3-MC. Twenty-four hr after 3-MC feeding, the rats were killed. Assays of BP hydroxylase.

Effect of Sequential Administration of Aromatic Hydrocarbons on BP Hydroxylase

On the basis of the supposition that the administration of many aromatic hydrocarbons that induce the synthesis of metabolizing enzymes in the liver and many other sites can result in the metabolic inactivation of subsequent doses of DMBA, experiments with sequential administration of protective aromatic hydrocarbons and DMBA were carried out. In these experiments, rats received a single feeding of a protective hydrocarbon, followed by a single dose of 30 mg of DMBA 24 hr later. The rats were killed 24 hr after the feeding of DMBA, and the liver, intestines, and adrenals were removed for enzyme assay. Each group contained 5 rats. For comparison, BP hydroxylase was also assayed in tissues from control groups in which the rats received only a single aromatic hydrocarbon.

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TABLE 1

Effect of Aromatics on Benzpyrene (BP) Hydroxylase Activity in Liver and Protection of Adrenal Cortex

<table>
<thead>
<tr>
<th>Dose of hydrocarbons (mg)</th>
<th>BP-Hydroxylase activity (units)</th>
<th>Adrenal necrosis and hemorrhagea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean ± S.D.</td>
</tr>
<tr>
<td>Control</td>
<td>37-56</td>
<td>45 ± 8</td>
</tr>
<tr>
<td>3-Methylcholanthrene</td>
<td>103-133</td>
<td>120 ± 20</td>
</tr>
<tr>
<td>5 mg</td>
<td>235-310</td>
<td>260 ± 31</td>
</tr>
<tr>
<td>Fluorene</td>
<td>900-1510</td>
<td>1230 ± 260</td>
</tr>
<tr>
<td>25</td>
<td>28-65</td>
<td>43 ± 20</td>
</tr>
<tr>
<td>100</td>
<td>39-62</td>
<td>48 ± 11</td>
</tr>
<tr>
<td>1,2-Benzofluorene</td>
<td>91-123</td>
<td>108 ± 11</td>
</tr>
<tr>
<td>5 mg</td>
<td>122-240</td>
<td>180 ± 40</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>75-156</td>
<td>100 ± 51</td>
</tr>
<tr>
<td>25</td>
<td>108-153</td>
<td>129 ± 30</td>
</tr>
<tr>
<td>100</td>
<td>64-133</td>
<td>103 ± 10</td>
</tr>
<tr>
<td>Chrysene</td>
<td>136-158</td>
<td>148 ± 10</td>
</tr>
<tr>
<td>5 mg</td>
<td>38-58</td>
<td>47 ± 7</td>
</tr>
<tr>
<td>100</td>
<td>53-120</td>
<td>80 ± 11</td>
</tr>
</tbody>
</table>

* No. of animals with adrenal necrosis/total no. of animals.

Table 1 shows the results of experiments with sequential administration of protective aromatic hydrocarbons and DMBA. The results suggest that an earlier protective dose of either 3-MC or DMBA did not cause the challenger DMBA to be inactivated metabolically so as its capacity to induce BP hydroxylase synthesis in the liver and intestines was concerned. It should be noted, however, that the values for the combinations of 2 hydrocarbons were less than additive if 10 mg of 3-MC instead of 30 mg of DMBA were given 2nd, in the sequence.

Influence of Ethionine on 3-MC-induced Increases in BP Hydroxylase and Protection of the Adrenal Cortex

There were 6 groups of 5 rats each in experiments concerning 3-MC-induced synthesis of BP hydroxylase and the influence of ethionine on the process. A dose of 3 mg of 3-MC was administered p.o., and 25 mg of ethionine were given i.p. 8, 4, or 0.5 hr either before or after the feeding of 3-MC. Twenty-four hr after 3-MC feeding, the rats were killed. Assays of BP hydroxylase.

Effect of Sequential Administration of Aromatic Hydrocarbons on BP Hydroxylase

On the basis of the supposition that the administration of many aromatic hydrocarbons that induce the synthesis of metabolizing enzymes in the liver and many other sites can result in the metabolic inactivation of subsequent doses of DMBA, experiments with sequential administration of protective aromatic hydrocarbons and DMBA were carried out. In these experiments, rats received a single feeding of a protective hydrocarbon, followed by a single dose of 30 mg of DMBA 24 hr later. The rats were killed 24 hr after the feeding of DMBA, and the liver, intestines, and adrenals were removed for enzyme assay. Each group contained 5 rats. For comparison, BP hydroxylase was also assayed in tissues from control groups in which the rats received only a single aromatic hydrocarbon.

The results illustrated in Chart 2 show that there was a greater increase in BP hydroxylase in the liver in rats receiving 2 hydrocarbons in sequential administration than in rats receiving a single hydrocarbon. Rats that received 3-MC + DMBA or DMBA + DMBA had an increase in BP hydroxylase far above that obtained in the liver in rats that received only 3-MC or DMBA. The increase resulting from these 2 sequential feedings of a protector and a challenger (DMBA) actually was an additive sum of the 2 single effects. The data for BP hydroxylase in the intestine were similar. The results suggest that an earlier protective dose of either 3-MC or DMBA did not cause the challenger DMBA to be inactivated metabolically so as its capacity to induce BP hydroxylase synthesis in the liver and intestines was concerned. It should be noted, however, that the values for the combinations of 2 hydrocarbons were less than additive if 10 mg of 3-MC instead of 30 mg of DMBA were given 2nd, in the sequence.

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There were 6 groups of 5 rats each in experiments concerning 3-MC-induced synthesis of BP hydroxylase and the influence of ethionine on the process. A dose of 3 mg of 3-MC was administered p.o., and 25 mg of ethionine were given i.p. 8, 4, or 0.5 hr either before or after the feeding of 3-MC. Twenty-four hr after 3-MC feeding, the rats were killed. Assays of BP hydroxylase.
EFFECT OF ETHIONINE ON INHIBITION OF INDUCTION OF ADRENAL HYDROXYLASE ACTIVITY

CHART 3.—At zero hr, 2 groups of rats received p.o. feedings of 3-MC, 1 group was given, in addition, ethionine, 25 mg i.p. BP hydroxylase activity was measured at 0.5, 4, and 8 hr before or after 3-MC feeding. *BP hydroxylase activity was calculated as % of 3-MC-induced activity at corresponding times.

TABLE 2

<table>
<thead>
<tr>
<th>3-MC dose (mg)</th>
<th>Ethionine dose (mg)</th>
<th>Adrenal necrosis/total no. of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>25</td>
<td>0/10 3/10 9/10 9/10 0/10 2/10 2/10 0/10</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>0/10 1/10 8/10 9/10 9/10 2/10 2/10 0/10</td>
</tr>
</tbody>
</table>

* On day 1, ethionine was injected, before and after 3-MC fed at zero hours, DMBA 30 mg fed p.o. on day 2 or 24 hours after 3-MC. Rats were killed and adrenals were studied on day 5 or 72 hr after DMBA administration.

+ 3-Methylcholanthrene.

+ Listed according to time of injection of ethionine related to 3-MC.

were carried out in liver, intestines, and adrenals. Each experimental group was paralleled by a control group in which only 3-MC was given.

A dose of 25 mg of ethionine given 0.5 hr preceding or following 3-MC administration almost completely nullified the stimulatory effect of 3-MC on induction of BP hydroxylase synthesis (Chart 3). At 4 hr either before or after 3-MC feeding, ethionine still significantly inhibited 3-MC-induced enzyme synthesis. A progressive decrease in the effect of ethionine on 3-MC-induced increases in BP hydroxylase in liver and intestines was observed as the time interval between 3-MC feeding and ethionine injection lengthened. It is interesting to note that the suppressive action of ethionine on enzyme synthesis is consistently more effective when ethionine is given before 3-MC feeding. The present data also suggest that the blocking effect of ethionine is quantitatively related to the dose of the inducer 3-MC. The blocking effect became less effective when the dose of 3-MC was increased without similarly increasing the dose of ethionine (Chart 3).

CHART 4.—The effect of actinomycin D and puromycin on 3-MC-induced stimulation of BP hydroxylase activity in rat liver. The numbers below each experiment represent the hr dose schedule of the inhibitor administration. In each experiment 3-MC was given p.o. at zero time, and rats were killed at the designated time as indicated by X. In rats receiving actinomycin D the schedule was as follows: 20 mg of actinomycin D was injected i.p. at -2, 0, and 2 hr. In animals receiving puromycin the dose schedule was the same. The dose of puromycin was 3 mg given i.p.

Does ethionine block aromatic-induced protection of the adrenal cortex against injury by DMBA? In the present study, the protective activity of 3-MC was almost entirely abolished by ethionine given 0.5 hr before or after the administration of 3-MC. This effect was greatly reduced, however, when ethionine was given 4 hr before or after 3-MC feeding. From there on, ethionine was no longer capable of blocking the protective effect of 3-MC (Table 2). Even so, it should be noted that 3-MC-induced synthesis of BP hydroxylase was still greatly depressed by ethionine at 4 hr before or after 3-MC feeding.

Effects of Actinomycin D and Puromycin on 3-MC-induced Increases in BP Hydroxylase and Protection of the Adrenal Cortex

Charts 4 and 5 show the increases that occurred in BP hydroxylase activity in the liver and intestines and the decrease that occurred in the enzyme activity in the adrenals after the administration of 3-MC. In the liver (Chart 4), both actinomycin D and puromycin inhibited 3-MC stimulation of BP hydroxylase synthesis if tissues were harvested for enzyme assays 6 hr after 3-MC administration. On the other hand, the stimulatory effect of 3-MC was no longer inhibited by either actinomycin D or puromycin if BP hydroxylase was determined 24 hr after 3-MC administration or 22 hr after the last dose of either of the 2 inhibitors. In the intestines (Chart 5), the stimulatory effect of 3-MC on BP hydroxylase activity was not apparent 6 hr after 3-MC feeding, but a 2- to 3-fold increase in the enzyme activity
Protection of Adrenal Cortex against DMBA Injury. I

CHART 5.—The effect of actinomycin D and puromycin on 3-MC-induced BP hydroxylase activity in the rat intestine. The numbers below each experiment represent the hr dose schedule of the inhibitor administration. X indicates time when rats were killed and tissues were removed for enzyme assays. The conditions for the experiment are the same as in Chart 4.

CHART 6.—The effect of actinomycin D and puromycin on 3-MC-induced BP hydroxylase activity in the rat adrenal. The numbers below each experiment represent the hr dose schedule of the inhibitor administration. X indicates time when rats were killed and tissues were removed for enzyme assays. The conditions for the experiment are the same as in Chart 4.

TABLE 3

<table>
<thead>
<tr>
<th>Compounds*</th>
<th>Dose 3-MC (mg)</th>
<th>Interval between last dose of actinomycin D or puromycin and DMBA † (hr)</th>
<th>Adrenal hemorrhage and necrosis ‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinomycin D, 25 µg X 3</td>
<td>3</td>
<td>6</td>
<td>0/10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>0/10</td>
</tr>
<tr>
<td>Puromycin, 3 mg X 3</td>
<td>3</td>
<td>0</td>
<td>10/10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>0/10</td>
</tr>
</tbody>
</table>

* Actinomycin D or puromycin was given 2 hr before, simultaneously with feeding of 3-MC, and 2 hr after 3-MC administration.
† All rats were killed 72 hr after the single feeding of 30 mg DMBA.
‡ No. of rats with hemorrhage and necrosis/total no. of rats.

in the intestinal mucosa was obtained 24 hr after 3-MC administration. Actinomycin D or puromycin induced only a slight inhibition of the stimulatory effect of 3-MC on BP hydroxylase activity in the intestines.

In the adrenal gland, both actinomycin D and puromycin appeared to have an additive effect on 3-MC inhibition of BP hydroxylase synthesis (Chart 6).

Actinomycin D did not block 3-MC protection of the adrenal cortex against injury by DMBA, even though it inhibited the stimulatory effect of 3-MC on BP hydroxylase activity. Results in Table 3 show that actinomycin D given 3 times at 2 hr before, simultaneously with, and 2 hr after 3-MC administration had no influence on the protective effect of 3-MC on the adrenals. In rats receiving actinomycin D in addition to 3-MC, the adrenals appeared grossly normal, and the cortical cells showed no necrosis microscopically.

In contrast, puromycin given in exactly the same time schedule effectively blocked 3-MC protection of the adrenal cortex if DMBA was given 6 hr after 3-MC administration (Table 3). On the other hand, in rats receiving DMBA 24 hr after 3-MC administration or 22 hr after the last dose of puromycin, the adrenals were yellow since 3-MC had induced protection.

Discussion

Drugs or chemicals with widely different structures that are foreign to the organism can induce the synthesis of enzymes that participate in the metabolic inactivation of those substances. Among the substances inducing the synthesis of drug-metabolizing enzymes are polycyclic aromatic hydrocarbons. Conney and Miller (3) have demonstrated that administration of a small dose of a polycyclic aromatic hydrocarbon to rats induces a marked increase in the synthesis of hydroxylating enzymes in the liver. These enzymes are localized in the microsomes, and their activity requires NADPH and oxygen. Induction of the synthesis of hydroxylating enzymes can be inhibited by simultaneous injection of actinomycin D (12, 13) or puromycin (2, 13). The presence of BP hydroxylase in other tissues, such as the intestines, kidneys, lungs, and adrenals, has subsequently been reported (10, 13, 21). These studies suggest that tissues exhibiting enzyme...
activity may represent physiologic sites for the hydroxylation of polycyclic aromatics.

Results of this study show that the normal mammary gland contains very small amounts (1-3 units/mg wet tissue) of BP hydroxylase. Administration of 3-MC did not induce stimulation of BP hydroxylase activity in this tissue. It would appear that the mammary gland does not play any significant role in the hydroxylation of aromatic hydrocarbons in mammary tissue. Most noteworthy is the observation that the mammary gland and adrenals are not responsive to 3-MC or other agents so far as the stimulation of BP hydroxylase synthesis is concerned. In the adrenals, in fact, suppression of enzyme activity was observed. These findings suggest that the mammary glands and adrenals are unable, under these circumstances, to inactivate aromatic hydrocarbons accumulating in them.

Dao et al. (7) made quantitative studies of tissue concentrations of 3-MC after a single large dose of the carcinogen. Twenty-four hr after p.o. administration of 3-MC to rats, the hydrocarbon was found mainly in the fatty and breast tissues; levels in other tissues, including the intestines, lungs, and kidneys, were either very small or insignificant. Interestingly, these are also the tissues which not only contain BP hydroxylase but are also sensitive to the stimulatory effect of the hydrocarbons in the induction of enzyme synthesis.

It is conceivable that the absence of any appreciable concentration of the ingested 3-MC in these tissues can be attributed to the enzymatic hydroxylation of 3-MC in these tissues. In the mammary glands and adrenals, BP hydroxylase activity is not stimulated, hence the 3-MC remains unmetabolized and accumulates. The fact that mainly the mammary glands and adrenals in rats treated with polycyclic hydrocarbons suggest that the carcinogenic agent is the unchanged hydrocarbon, rather than the hydroxylated metabolites.

The mechanism by which aromatic polycyclic hydrocarbons protect the adrenal cortex against injury from DMBA is not understood. It is now known that substances of diverse chemical natures, such as aromatic hydrocarbons (6, 16), aromatic azo dyes (19), amphenone analogs (4, 9), and phenothiazines (20), are effective adrenal protectors. Among these compounds many are effective inducers of drug-metabolizing enzymes, but some have no effect on the induction of enzyme synthesis.

The present study was intended to gain insight into the problem whether aromatic induction of the synthesis of hydroxylating enzymes is one of the factors contributing to the effect of aromatic hydrocarbons in protecting the adrenal cortex against injury by DMBA. Sequential administration of 3-MC + DMBA and DMBA + DMBA induced the synthesis of amounts of BP hydroxylase larger than the sums of the amounts synthesized after corresponding single doses. These data seem to suggest that large amounts of DMBA are not rapidly inactivated by BP hydroxylase as a result of a previous treatment with 3-MC. Huggins and Fukunishi (17) reported similar observations on measuring menadione reductase in the liver after treatment with 3-MC and DMBA. These authors found that rats that received 3-MC + DMBA had a far greater increase in hepatic menadione reductase (376%) than did rats that received 3-MC alone (276%).

The results of the present study do not seem to support the hypothesis that markedly increased amount of hydroxylating enzymes in the liver and intestines, following the administration of aromatic polycyclic hydrocarbon, makes possible the metabolic inactivation of DMBA before it can reach the adrenal cortex.

Other alternative interpretations of our data must, however, be considered. It is conceivable that increased hydroxylase activity may indeed play a role in DMBA destruction, but it might not necessarily be complete or instantaneous and thereby the unmetabolized DMBA can still be an inducer. It should also be pointed out that in the present investigation the experiments involve the study of an in vivo effect of DMBA on adrenal cortex after the administration of another polycyclic hydrocarbon earlier as an enzyme inducer. Evidence to show that the enzyme systems hydroxylation the 2 polycyclic hydrocarbons are actually parallel is, however, lacking.

In the present study, ethionine inhibited the 3-MC-induced increase in BP hydroxylase activity when the amino acid was given 0.5 hr before or after 3-MC. Only a slight increase in BP hydroxylase activity (5-10%) was observed when ethionine was given at 4 hr. Ethionine effectively blocked the protective action of 3-MC at 0.5 hr; but administration of the amino acid 4 hr prior or subsequent to 3-MC had no effect on 3-MC-induced protection of the adrenal cortex against injury by DMBA.

In similar experiments with actinomycin D and puromycin, however, the results were different. Whereas both actinomycin D and puromycin effectively inhibited the 3-MC-induced increase in BP hydroxylase at 6 hr, only puromycin abolished 3-MC-induced protection of the adrenal cortex against injury by DMBA. Actinomycin D was completely ineffective. A clear interpretation of these conflicting results is not possible at this time. The data with actinomycin D, nonetheless, provides us with some evidence that large doses of DMBA are not rapidly inactivated or metabolically destroyed as a result of increases in BP hydroxylation synthesis induced by 3-MC, since actinomycin D, an inhibitor of DNA-dependent RNA synthesis (14), blocks 3-MC stimulation of BP hydroxylase synthesis but is unable to abolish the adrenal-protective effect of 3-MC.

What about ethionine and puromycin, which both inhibit 3-MC-induced stimulation of the synthesis of BP hydroxylase and both effectively block the 3-MC-induced protection of the adrenal cortex against injury by DMBA? Huggins and Fukunishi (17) demonstrated that DMBA caused a greatly reduced incorporation of tritium from thymidine-3H into washed acid-insoluble residue of the adrenal. 3-MC given prior to DMBA increased considerably the amount of tritium which was incorporated. It suggested that 3-MC mitigated the drastic effect of DMBA on DNA synthesis. These authors (17) postulated that induction of adrenal hemorrhage by DMBA depends on the extent of damage to DNA. Aromatic protectors stimulated protein synthesis, which in turn improved DNA synthesis, hence the mitigation of the damaging effect of large doses of DMBA. It is well established that ethionine and puromycin are inhibitors of protein synthesis (11, 15, 22). The present study seems to support the postulate that protection of adrenals is an effect that requires induction of protein synthesis. However, another possibility exists that these inhibitors may act directly on the adrenals and produce necrosis, an effect that may or may not be related to their inhibitory action on protein synthesis.

Acknowledgment

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References


On the Mechanism of Inducing Protection of the Adrenal Cortex against Injury from 7,12-Dimethylbenz(a)anthracene: I. Effects of Inducers on Benzpyrene Hydroxylase Activity

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