Effects of Polynuclear Aromatic Hydrocarbons on Benzpyrene Hydroxylase Activity in Rats.* (29446)

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Recently Dao and Tanaka reported that adrenal apoplexy induced in rats by 7,12-dimethylbenz(a)anthracene (DMBA) could be prevented by pretreating the rats with any of a number of polynuclear aromatic hydrocarbons(1,2). This interesting observation has since been confirmed by other workers (3).

The mechanism by which the polynuclear aromatic hydrocarbons protect the adrenal cortex against injury from DMBA is not understood. Some evidence suggests that adrenal apoplexy is hormone-dependent(4-6). The susceptibility of the adrenal cortex to DMBA appears to be directly related to the synthetic capacity of the gland.

It has been well established since the early work of Conney and Miller(7) that administration of a small dose of a polynuclear hydrocarbon to rats induces a marked increase in hydroxylating enzymes in the liver. More recently it has been shown that the gastrointestinal tract, lungs and kidneys are also sites where hydroxylating enzymes increase after injection of polynuclear hydrocarbons (8,9). It is conceivable that markedly increased concentrations of hydroxylating enzymes in the liver and other sites following pretreatment with polynuclear hydrocarbons make possible the metabolic destruction of DMBA before it can reach the adrenal cortex in sufficient quantity to inflict damage upon the gland. To test the validity of such a hypothesis, benzpyrene hydroxylase in the liver and adrenal glands of rats receiving polynuclear aromatic hydrocarbons was studied.

Materials and methods. Female Sprague-Dawley rats, 55 to 60 days old and weighing 160 to 175 g, were fed a commercial ration (Rockland diet) and were given water *ad lib.* Each experimental group had 5 rats, and was paralleled by a control group of 5 untreated rats. All hydrocarbons studied were dissolved in olive oil, and rats were fed a single dose of the solution. The animals were killed by decapitation at 4, 24, 48 and 72 hours after the feeding of the hydrocarbon, and a piece of liver (approximately 20 to 30 mg) and the adrenal glands were excised and weighed.

A 0.25% homogenate of each liver and 1% homogenate of each pair of adrenals were made in cold (0°-2°C) isotonic KCl solution with a Potter-Elvehjem homogenizer with a Teflon pestle. All tissues were homogenized and assayed individually.

Assays for benzpyrene hydroxylase (BP hydroxylase) were performed essentially according to the method described by Wattenberg *et al*(8) who used 1 ml of a reaction mixture containing 1 mg/ml NADPH (TPNH), 0.5 mg/ml NADH (DPNH), 0.06 M nicotinamide, 0.05 M KCl, and 0.05 M pH 7.4 sodium phosphate buffer with 2 ml of tissue homogenate. In our own assays, however, instead of using NADPH, 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 was used. To the reaction mixture 50 μg of benzo(a)pyrene in 0.1 ml of acetone was added. The mixture was incubated with shaking for 10 minutes at 37°C, and the reaction was stopped by adding 3.0 ml of cold acetone. The 9 ml of heptane (Spectroanalyzed, b.p. 98.4°C) was added to the mixture, and the flasks were incubated for 10 minutes at 37°C to facilitate quantitative extraction of the metabolite into the organic solvent phase. The mixture was stored in a dark coldroom at 4°C for 24 hours. An aliquot of 5 ml of the organic phase (top layer) was removed, and was extracted with 5 ml of 1 N NaOH.

The fluorescence of the aqueous extract

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was determined in a Farrand fluorometer, Model A, equipped with a primary filter that transmits light maximally at 390 mμ and a secondary interference filter with a peak wavelength of 525 mμ and a half band width of 14 mμ. Quinine sulfate solution (0.4 μg/ml in 1 N H_2SO_4) was used as a fluorescent standard in each experiment. Fluorescence was standardized with 8-hydroxybenzpyrene in the experiments of Wattenberg *et al*(7), but not in our own. Enzyme activity was calculated as fluorescence per mg of tissue. Zero-time control values for the tissue and incubation mixture were subtracted from the values for the incubated flasks. In our experiments the zero-time values ranged from 0 to 0.5 galvanometer divisions per mg of tissue.

In another experiment, 10 mg of 2-methyl-1,2-bis-(3-pyridyl)-1-propanone (metopirone, or SU 4885) in 0.1 ml of olive oil was given intraperitoneally for 2 doses at an interval of 4 hours, to 2 groups of 5 rats each. Each experimental group was paralleled by an untreated control group. The rats were killed 4 hours (Group 1), or 24 hours (Group 2) after injection of the second dose of metopirone. Liver and adrenals were assayed for BP hydroxylase activity.

Results. The level of BP hydroxylase is expressed as fluorescence per mg of tissue. Changes of enzyme activities in the tissues after treatment are calculated as percent of controls.

BP hydroxylase activity in the adrenal glands of 250 control rats range from 11.3 ± 1.2 to 18.3 ± 1.9 with a median of 15.8 and a mean of 15.3 ± 4.5. The activity in the livers of the identical control groups range from 10.1 ± 1.7 to 18.6 ± 2.8 with a median of 13.4 and a mean of 13.5 ± 1.9. It appears that the levels of enzyme activity in adrenals and liver are similar.

A single feeding of 30 mg of 3-MC increased the BP hydroxylase activity in the liver markedly. The activity reached its peak within 48 hours (Fig. 1). In contrast, the BP hydroxylase activity in the adrenal glands 24 to 48 hours after 3-MC feeding was depressed by 50%. It is interesting to note that the decrease of BP hydroxylase activity in

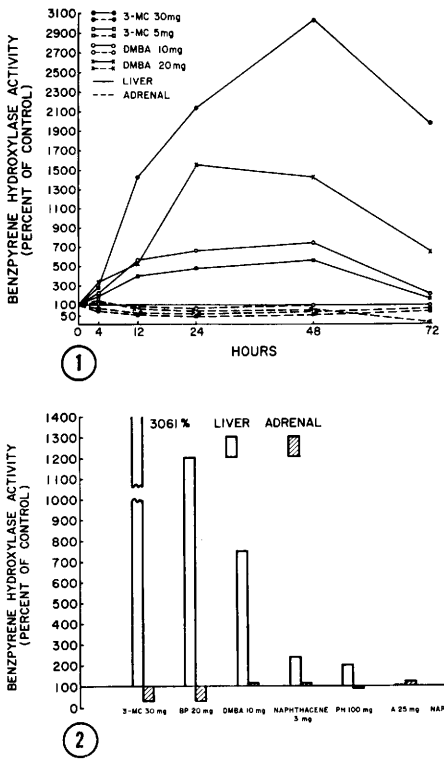

FIG 1. Effect of a single feeding of 3-MC or DMBA on BP hydroxylase activity in liver and adrenals in rats.

FIG. 2. BP hydroxylase activity in the liver and adrenals in rats 48 hours after feeding a single dose of hydrocarbon.

the adrenal is consistent throughout the experiment. Results following intraperitoneal injection of 3-MC were similar to those after 3-MC feeding.

DMBA feeding similarly increased the BP hydroxylase activity in the liver, but to a lesser extent. Enzyme activity showed a dose-response effect (Fig. 1); whereas 10 mg of DMBA increased the BP hydroxylase in the liver only slightly, 20 mg of the hydrocarbon induced a marked increase in the enzyme level. The dose-response effect was similarly observed with 3-MC. The increase of BP hydroxylase activity in liver was nearly 5 times more in rats receiving 30 mg of 3-MC than in those given 5 mg of 3-MC only. In contrast to 3-MC, DMBA is not as effective in inhibition of enzyme activity in the adrenals. In some animals in groups given 20 mg of DMBA, adrenal necrosis occurred; the much-decreased enzyme activity in the adre-

nals in the 72-hour group was due to necrosis.

The effects of several polynuclear aromatic hydrocarbons on BP hydroxylase activity in the liver and adrenals were compared, using doses optimal for 100% protection of the adrenal cortex against injury from DMBA(2). (Naphthalene was totally ineffective as a protector at any of the dose levels studied, hence a dose of 150 mg was chosen arbitrarily.)

3-MC, benzo(a)pyrene (BP) and DMBA are 3 potent inducers of BP hydroxylase in the liver, but enzyme activity is only slightly increased by phenanthrene (PH) and unaffected by anthracene (A). Naphthacene, although an effective protector, causes only slight increase of enzyme level in the liver (Fig. 2).

Metopirone, an inhibitor of 11β-hydroxylation of adrenal cortical steroid hormones, increased the BP hydroxylase level in liver slightly 4 hours (210% of control) and 24 hours (287% of control) after administration of the compound. Unlike the hydrocarbons, metopirone appears to have a stimulatory effect on BP hydroxylase activity in the adrenals. The enzyme activities are increased 4 hours (163% of control) and 24 hours (138% of control) after administration of metopirone.

Discussion. The presence of BP hydroxylase in rat adrenals was demonstrated earlier by a histochemical method, but quantitative assays of the enzyme in the adrenals have not been reported previously(10). Results obtained in the present investigation show that BP hydroxylase activity in normal rat adrenals is approximately equal to the activity in liver.

Most of the substances effective in protecting the adrenal cortex from DMBA-induced necrosis increase the BP hydroxylase in the liver; but phenanthrene and anthracene, although effective inhibitors of adrenocortical necrosis, have little or no effect on BP hydroxylase activity. Naphthalene, an ineffective protector, induced a slight increase of BP hydroxylase (150%). Thus it appears that the effect of the hydrocarbons on BP hydroxylase is not always related to their protective activities.

It is well known that the adrenal cortex is rich in steroid-hydroxylating enzymes. Whether there is much difference in the substrate specificity of these two types of hydroxylating enzymes is not entirely clear. Metopirone, an effective inhibitor of 11β-hydroxylation of adrenal cortical steroid hormones evidently does not inhibit the BP hydroxylase. On the contrary, metopirone seems to have some slight stimulatory effect on BP hydroxylase in the adrenals. It is quite evident from the results of this study, however, that the adrenals are not an important site for the hydroxylation of aromatic hydrocarbons.

Summary. BP hydroxylase in liver and adrenals was quantitatively estimated in rats pretreated with polynuclear aromatic hydrocarbons. Whereas 3-MC, BP and DMBA markedly stimulated BP hydroxylase activity in the liver, they depressed the enzyme activity in the adrenal glands. These compounds were also effective protectors of the adrenal cortex against injury by DMBA. Phenanthrene and anthracene had little or no effect on BP hydroxylase in the liver, but they were effective protectors at the dose levels studied. Metopirone, an inhibitor of 11β-hydroxylation of adrenal cortical steroid, did not inhibit the hydroxylating enzymes of BP. Instead, it appeared to stimulate BP hydroxylase in the adrenal glands.

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