The Effects of Cigarette Smoke on the Metabolism of [³H]Benzo(a)pyrene by Rat Lung Microsomes¹

Pekka Uotila, Olavi Pelkonen,² and Gerald M. Cohen³

Department of Physiology, University of Turku, FIN-20520, Turku 52, Finland [P. U.], Department of Pharmacology, University of Oulu, FIN-90220, Oulu 22, Finland [O. P.], and Department of Biochemistry, University of Surrey, Guildford, GU2 5XH, Surrey, England [G. M. C.]

SUMMARY

The metabolism of [³H]benzo(a)pyrene and the activities of specific enzymes involved in its further metabolism were studied in lung microsomes from sham- and cigarette smoke-exposed and 3-methylcholanthrene-pretreated rats. Benzo(a)pyrene was converted into metabolites cochromatographing with reference dihydrodiols, phenols, and quinones as well as some unknown metabolites. Exposure of rats to cigarette smoke increased the formation of different metabolites from 3- to 6-fold, whether the exposure was for 1, 10, or 21 days. The metabolite patterns were similar in smoke-exposed and 3-methylcholanthrene-pretreated rats, but 3-methylcholanthrene pretreatment caused a greater increase in metabolite production.

Pulmonary aryl hydrocarbon hydroxylase activity was increased from 3- to 6-fold after smoke exposure. The activity of epoxide hydratase (substrate: styrene oxide) was decreased after 1 day of smoke exposure and did not change after exposure for 10 or 21 days. Glutathione S-transferase activity (substrate: styrene oxide) increased after 1 and 10 days of smoke exposure. No significant changes could be seen in the activity of uridine diphosphate glucuronosyltransferase (substrate, 4-methylumbelliferone).

Isolated perfused rat lungs and lung microsomes convert benzo(a)pyrene to similar metabolites, but differences were observed in the absolute and relative amounts of different metabolites. 9,10-Dihydro-9,10-dihydroxybenzo(a)pyrene and 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene were the major dihydrodiols formed in perfusion experiments, whereas 4,5-dihydro-4,5-dihydroxybenzo(a)pyrene was the major metabolite in microsomes. This difference was probably due to differing rates of further conjugation of primary metabolites in the isolated perfused lungs.

INTRODUCTION

BP⁴ belongs to a large group of PAH, which are abundantly present in the environment (12). Many PAH, including BP, are carcinogenic in experimental animals and probably also in humans. Especially important is the relationship between PAH and bronchogenic carcinoma in man. Cigarette smoke is the single most important factor in the etiology of lung cancer, and because cigarette smoke contains large amounts of PAH, especially BP, it is possible that PAH are responsible for the carcinogenic action of cigarette smoke.

On the basis of animal experiments it seems probable that the carcinogenic actions of BP and many other chemical carcinogens are related to specific aspects of their metabolism (20, 23, 29, 39). Several different metabolic intermediates have been suggested as the ultimate carcinogenic forms of BP, and much recent evidence implicates the possible importance of certain diol-epoxide intermediates, in this respect (13, 16, 22, 33, 36, 40, 49). Whatever will prove to be the ultimate carcinogenic form or forms, the elucidation of the metabolic fate of BP must be 1 of the 1st steps in understanding its carcinogenicity.

There are some studies, mostly in vitro, concerning the fate of BP in lungs. It has been shown that exposure of animals to cigarette smoke increases the pulmonary activity of AHH, an enzyme metabolizing BP (1, 41, 45, 47, 48), and also that AHH activity in lung macrophages is higher in humans who smoke cigarettes than in nonsmokers (9). There are also some studies on the covalent binding of BP in human bronchus (16, 18). However, these studies cannot give reliable information on the fate of BP in intact lungs. More recently, a few studies on the metabolism of BP in isolated perfused lung preparations have appeared (10, 44, 46).

In the previous paper (11), we investigated the effect of cigarette smoke on the metabolism of BP in isolated perfused rat lung. In order to elucidate the possible correlation between perfusion and in vitro experimental systems, we studied the effects of cigarette smoke on both the metabolite pattern of BP and enzymes involved in its further metabolism in rat lung microsomes. The final goal was to clarify whether the in vitro metabolism and enzyme activities in any way represent the metabolism of the compound measured in the isolated perfusion system.
MATERIALS AND METHODS

Materials. [3H]BP (generally labeled; specific activity, 20 Ci/mmole) was purchased from the Radiochemical Center, Amersham, England, and was purified by the method of De Pierre et al. (14). [3H]BP in acetone was diluted with unlabeled BP (Sigma Chemical Co., St. Louis, Mo.) to the appropriate concentration. Synthetic BP derivatives were either generous gifts from Dr. H. V. Gelboin (National Cancer Institute, Bethesda, Md.) or prepared by the method of Sims (38). The identity of the metabolites was confirmed by UV spectroscopy. NADP was purchased from Boehringer Mannheim, Mannheim, Germany; isocitric acid and isocitrate dehydrogenase were from Sigma Chemical Co. All other compounds used were of reagent grade.

Preparation of Microsomes. Lungs were rinsed in ice-cold 0.25 M sucrose and homogenized in 4 volumes of 0.25 M sucrose with an Ultra-Turrax cutting-blade homogenizer. The CaCl2-precipitated microsomes were isolated and washed as described earlier (43) according to the modified procedure of Kamath et al. (24).

Protein determinations were carried out by the biuret method using bovine serum albumin (Armour Pharmaceuticals, Eastbourne, England) as a standard (26). The protein content of the microsomes and the soluble fraction of the cells remaining after precipitation of the microsomes were 13.1 ± 0.8 and 105 ± 2 mg/g, respectively (n = 12). The soluble fraction was used to measure the activity of GT.

Metabolism of BP in Vitro by Lung Microsomes. The incubation mixture consisted of 0.5 ml of cofactor mixture (0.25 mM NADP, 5 mM isocitrate, 10 units isocitrate dehydrogenase, 8 mM MgCl2, 40 mM KCl), microsomes (0.1 ml corresponding to 100 mg of lung wet weight), 1.0 ml of 0.1 M potassium phosphate buffer (pH 7.4), and 0.4 ml of distilled water to make the final volume 2 ml. After a 2-min preincubation at 37°, the reaction was started with the addition of BP (1.0 nmole, about 106 cpm of [3H]BP) in 25 µl of acetone. Complete incubation mixtures incubated at 4° were used as blanks. After 30-min incubation in a shaking water bath at 37°, the reaction was stopped with 4 ml of cold ethanol. The reaction mixture was extracted twice with cold ethyl acetate and centrifuged at 10000 x g for 3 min; the organic phases were combined and dried with Na2SO4, reduced to dryness with a stream of N2, and the residue was dissolved in a small volume of ethyl acetate containing unlabeled reference compounds. The residue was spotted onto a thin-layer chromatography plate and run in a solvent system of benzene:ethanol (9:1, v/v). Fluorescent spots were marked under UV, the plate was cut into 5-mm bands which were put into scintillation vials, 10 ml of scintillation fluid were added, and samples were counted in a Packard Tri-Carb liquid scintillation counter. The metabolites were quantified as described in the preceding paper (11).

The incubation time of 30 min was chosen because it allowed a more reliable detection of BP metabolites. Preliminary experiments showed, particularly at higher enzyme concentrations, that not all the metabolites were produced linearly for 30 min. However, the amount of enzyme corresponding to 100 mg, wet weight, of lung resulted in an almost linear production of all metabolites for 30 min.

Assays for Enzyme Activities. The activity of AHH was determined fluorimetrically utilizing BP (30) as previously described (42). UDPGT activity was assayed fluorimetrically with 4-methylumbelliferone (British Drug House Chemicals, Poole, England) as the aglycone (2, 5). The EH activity was measured with [7-3H]styrene oxide (NEN Chemicals GmbH, Dreichenhain, West Germany) as substrate by a microassay developed by Oesch et al. (35) with minor modifications (41). GT activity was determined from the soluble fraction with [7-3H]styrene oxide by the method of Marniemi and Parkki (28).

RESULTS

[3H]BP (0.5 µM) was metabolized by lung microsomes from sham-, cigarette smoke-exposed, and 3-MC-pretreated rats to ethyl acetate-soluble metabolites that cochromatographed with references 9,10-dihydrodiol, 7,8-dihydrodiol, 4,5-dihydrodiol, 3-0H-BP, quinones, and unchanged BP as well as metabolites migrating just beyond the origin (Chart 1; Table 1). A metabolite (Y) migrating between 4,5-dihydrodiol and 3-0H-BP was particularly evident after 3-MC pretreatment but was also present in some experiments using microsomes from smoke-exposed rats. The amount of BP metabolism and the metabolite patterns were similar after 1, 10, or 21 days of cigarette smoke exposure (Table 1; Chart 1). The pretreatment of rats with 3-MC increased the metabolism of BP by lung microsomes even more than the exposure to cigarette smoke, but the metabolite profile seemed to be qualitatively similar (Table 1).

Lung microsomes from rats exposed to cigarette smoke for 1, 10, or 21 days exhibited a significant increase in the production of all major metabolite fractions (Table 1). These major metabolite fractions accounted for most of the metabolism of BP in vitro, as seen by comparing the disappearance of BP from the incubation mixture and adding the major metabolite fractions together (Table 1). The extent of increase in metabolite formation in smoke-exposed rats was about 6-fold for phenols, from 4- to 6-fold for dihydrodiols, and from 2- to 4-fold for quinones (Table 1). 3-MC pretreatment caused a significant increase in metabolite production when compared to either sham- or cigarette smoke-exposed animals (Table 1). After 3-MC pretreatment, the increase in the amounts of the metabolites compared to the mean values of sham-exposed rats was 20-fold for phenols and dihydrodiols and about 10-fold for quinones (Table 1).

The activities of different enzymes involved either in the initial metabolism of BP (AHH) or the further metabolism of primary metabolites (EH, GT, and UDPGT) are shown in Table 2. Similar increases in pulmonary AHH activity were observed after cigarette smoke exposure of rats for either 1,
DISCUSSION

Several studies have shown that AHH activity in the lungs of rodents is increased by exposure to animals to cigarette smoke (14, 41, 45, 47, 48) or marijuana smoke (27) and by pretreatment with 3-MC (21). 3-MC pretreatment of animals when compared to values from sham-exposed rats caused significant increases in the activities of AHH and UDPGT but not of GT (Table 2).

In contrast to the large increase of AHH activity, only...
The effect of cigarette smoking and 3-MC pretreatment on the activities of AHH, EH, UDPGT, and GT in rat lungs

Rats were exposed to the smoke from 5 cigarettes daily for either 1, 10, or 21 days and killed 20 hr after the last exposure. Some rats were treated i.p. with 3-MC (20 mg/kg in corn oil) daily for 3 days and killed 20 hr after the last injection. Lung microsomes were isolated by 

<table>
<thead>
<tr>
<th>Enzyme (substrate)</th>
<th>1 day</th>
<th>10 days</th>
<th>21 days</th>
<th>3-MC pretreated</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHH (BP)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(pmoles/min/g lung)</td>
<td>4.09 ± 0.71*</td>
<td>11.5 ± 1.0b</td>
<td>2.18 ± 0.42</td>
<td>10.6 ± 1.0b</td>
</tr>
<tr>
<td>EH (styrene oxide)</td>
<td>2.28 ± 0.13</td>
<td>1.53 ± 0.10c</td>
<td>2.18 ± 0.31</td>
<td>2.25 ± 0.15</td>
</tr>
<tr>
<td>(pmoles/min/g lung)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UDPGT (methyl umbelliferone)</td>
<td>3.52 ± 0.50</td>
<td>3.88 ± 0.14</td>
<td>3.03 ± 0.31</td>
<td>3.00 ± 0.19</td>
</tr>
<tr>
<td>(pmoles/min/g lung)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GT (styrene oxide)</td>
<td>1180 ± 70</td>
<td>1580 ± 120d</td>
<td>910 ± 40c</td>
<td>1150 ± 40c</td>
</tr>
</tbody>
</table>

* Mean ± S.E. of 4 separate experiments.
* p < 0.001 that results are different from those of corresponding sham-exposed rats by Student's t-test.
* p < 0.01 that results are different from those of corresponding sham-exposed rats by Student's t-test.
* p < 0.05 that results are different from those of corresponding sham-exposed rats by Student's t-test.
* The number of experiments was 6.

Table 2

BP Metabolism in Lungs
diol produced was 9,10-dihydrodiol. The increase in all the dihydrodiols and phenols in the lung microsomes of 3-MC-pretreated rats is in agreement with the observations of Rasmussen and Wang (37).

When the rate of metabolism and the metabolite pattern of BP by lung microsomes are compared with those of isolated perfused rat lungs (11), some major quantitative differences are seen. This stresses the importance of maintaining the structural integrity of the lungs when trying to assess their metabolic capacity both quantitatively and qualitatively. The qualitative nature of the metabolites from BP formed by isolated perfused lungs (11) and lung microsomes were similar, in agreement with the findings of Law et al. (25), who found the same major metabolites formed from methadone, pentobarbital, and parathion using either perfusions or microsomes. However, metabolite production was greater with microsomal preparations (Table 1) than in perfused lungs (Ref. 11, Tables 3 and 4), in particular with 3-MC-treated animals, when rates of 9,10-dihydrodiol formation with microsomes and isolated perfused lung were 17.6 and 2.3 pmoles/min/g lung, respectively. The larger amounts of metabolites observed in the microsomal preparations may be due to several factors acting alone or in combination: (a) the differences in the further metabolism of primary metabolites to water-soluble conjugates; (b) the limitations of substrate penetration to the metabolizing enzymes; and (c) rate-limiting amounts of cofactors in the perfused lungs.

The relative amounts of dihydrodiols formed by either lung microsomes or by isolated perfused rat lungs (11) were different. 4,5-Dihydrodiol was the major dihydrodiol formed in microsomal experiments (Table 1), whereas more of both 7,8-dihydrodiol and 9,10-dihydrodiol than 4,5-dihydrodiol was formed by isolated perfused lungs (Ref. 11, Table 3). This difference was observed with both sham- and cigarette smoke-exposed as well as with 3-MC-pretreated rats. Similar differences of dihydrodiol production were also observed in the lung tissue as well as in the perfusate (Ref. 11, Tables 1 and 2). The most probable explanation for these differences is that with isolated perfused lungs, but not with microsomes, further metabolism of the products by conjugation reactions is possible. Glutathione and glucuronide conjugations cannot take place in microsomal preparations, because no glutathione, GT, or uridine diphosphogluconic acid is present, whereas reactions such as glucuronide conjugation are known to occur in isolated perfused lung (3). Our results showing relatively less 4,5-dihydrodiol than the other dihydrodiols with the isolated perfused lungs are consistent with the observations that (a) the K-region epoxide, benzo(a)pyrene 4,5-oxide, is conjugated more readily with glutathione by GT than non-K-region epoxides, benzo(a)pyrene 7,8- or benzo(a)pyrene 9,10-oxides (6, 19), and (b) 4,5-dihydrodiol is a better substrate for glucuronide conjugation than either 7,8-dihydrodiol or 9,10-dihydrodiol (32).

Thus, in conclusion, although the nature of the metabolites formed by lung microsomes and isolated perfused lungs were similar, important quantitative differences were observed in both the absolute and relative amounts of different metabolites.

ACKNOWLEDGMENTS

We thank our colleagues Dr. H. Vainio and Dr. J. Marniemi for helpful discussions. The skilful technical assistance of Tarja Laiho, Leena Ruokonen, and Raija Söderholm is gratefully acknowledged. The authors wish to thank Dr. Harry V. Gelboin (National Cancer Institute, Bethesda, Md.) for the generous gift of authentic metabolites of benzo(a)pyrene.

REFERENCES


P. Uotila et al.


The Effects of Cigarette Smoke on the Metabolism of $[^3\text{H}]$Benzo(a)pyrene by Rat Lung Microsomes

Pekka Uotila, Olavi Pelkonen and Gerald M. Cohen


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/37/7_Part_1/2156

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.