Effect of Ozone on Benzpyrene Hydroxylase Activity in the Syrian Golden Hamster

Michael S. Palmer, Donald H. Swanson, and David L. Coffin

Environmental Protection Agency, Air Pollution Control Office, Division of Health Effects Research, Cincinnati, Ohio 45237

SUMMARY

Exposure of Syrian golden hamsters to increasing concentrations of ozone decreases benzpyrene hydroxylase activity in the lungs as much as 70%, while the benzpyrene hydroxylase activity in the liver remains unchanged. The degree of inhibition is the same for both artificially induced high enzyme levels and normal, relatively low enzyme levels. Lung weight comparisons are used to show that dilution of the enzyme, secondary to edema formation, plays only a small part in the reductions in enzyme activity. Ozone may act as a cocarcinogen with inhaled benz(a)pyrene by delaying the enzymatic transformation of the hydrocarbon.

INTRODUCTION

In the middle and late 1930's, Chalmers (5, 6) with Crowfoot (7) described the elimination of BaP1 from rats in the form of a hydroxylated metabolite. Several years later, Berenblum et al. (2, 3), examining the feces of rats and mice inoculated i.p. with BaP, demonstrated an 8-hydroxy and a diquinone metabolite, neither of which had the carcinogenic potential of the parent compound (10).

Further studies (4) showed that an enzyme responsible for the conversion of BaP to its less carcinogenic metabolites is present in large amounts in the microsomal fraction of liver and that production of this enzyme can be induced by many substrates (9, 13) and other compounds (21, 22, 24, 25). The enzyme, benzpyrene hydroxylase, has now been detected in varying amounts of many organ systems and appears to be TPNH, DPNH, and oxygen dependent.

In 1968, Saffiotti et al. (17) reported a high incidence of pulmonary carcinomas in hamsters given serial intratracheal inoculations of BaP adsorbed to hematite (Fe2O3). The role of hematite in this system is uncertain at present, but in part it may prolong the residence of the carcinogen in the lung by inhibiting the hydroxylating enzyme (11). Sunderman (20) has shown that inhaled nickel inhibits the induction of benzpyrene hydroxylase, and other metals have also been shown to decrease its activity (11).

The possibility exists that many elements and compounds may act as cofactors in carcinogenesis by inhibiting enzymes that detoxify inhaled carcinogens. As part of a continuing investigation in this laboratory of the biological and biochemical effects of gaseous pollutants, especially ozone, Hurst et al. (15) have demonstrated the inactivation of 3 hydrolytic enzymes in pulmonary alveolar macrophages exposed to ozone in vitro and Hurst and Coffin (14) have shown this in vitro. If ozone acts as a cocarcinogen in the lung (19), as suggested, it is possible that one of the mechanisms of its action is the inhibition of detoxification enzymes.

The purpose of this study is to examine the direct effect of ozone on the activity in the lung of benzpyrene hydroxylase.

MATERIALS AND METHODS

All animals used in this study were randomly bred male and female Syrian golden hamsters (Lakeview Hamster Colony, Newfield, N. J.1), 5 to 10 weeks old at the time of sacrifice. Animals were selected randomly for ozone treatment, and a similar age and animal weight distribution existed in each treatment group. A room-air control animal was sacrificed and assayed with each exposed animal throughout the experiments.

All hamsters were sacrificed within 30 min after exposure to ozone by an i.p. injection of 100 mg pentobarbital sodium (Nembutol, Lilly Pharmaceuticals, Indianapolis, Ind.). The lungs were immediately removed en bloc and washed in iced 0.9% NaCl solution, and the left lower lobe was weighed and assayed. The use of a large dose of barbiturate enabled us to remove the lungs before the cessation of respiration. Enzyme levels obtained with this approach were not significantly different from those in a series of hamsters sacrificed by cervical fracture.

Ozone was generated by passing oxygen through a neon tube-silent arc generator. The resulting effluent was mixed with filtered room air and delivered to stainless steel animal exposure chambers (11.4 cu ft, exclusive of funnels) at a rate that ensured 1 complete chamber atmosphere change/min. Ozone concentrations were measured by a standard wet method (18) at 15-min intervals during each exposure. All exposures were for 3 hr.

The enzyme assay used was a modification of the procedure described by Wattenberg et al. (23). A 1.25% homogenate of the liver or lung specimen was prepared with cold, isotonic...
KCl with a Tri-R Stir-R model S63C homogenizer. Homogenate (2 ml) was added to each of 2 cold 25-ml Erlenmeyer flasks containing 50 μg BaP (Aldrich Chemical Co., Inc., Milwaukee, Wis.) in 0.1 ml cold acetone. To one flask was added 1 ml of reaction mixture which contained 1 mg TPNH, 0.5 mg DPNH (Sigma Chemical Co., St. Louis, Mo.), 0.06 M nicotinamide, 0.05 M KCl, and 0.05 M sodium phosphate buffer at pH 7.4. The 2nd flask, containing the same reaction mixture without TPNH, was used to determine background fluorescence. The samples were agitated in a 37° water bath for 5 min (liver) or 20 min (lung), after which the reaction was stopped with 3.0 ml of cold acetone. Petroleum ether (9.0 ml) was added to extract the hydroxylated BaP, and the flasks were agitated at room temperature in a covered shaker for 15 min, then stored in the dark at 4° for 24 hr. Then 1 ml (liver) or 5 ml (lung) of the organic phase was removed and extracted with 10.0 ml 1 N NaOH by inversion and rotary mixing. An aliquot of the aqueous phase was removed and placed in silica cuvets for fluorescence determinations.

Fluorescence was determined with a Perkin-Elmer MPF-2A fluorescence spectrophotometer. Hydroxybenzpyrene in dilute sodium hydroxide fluoresces maximally at a wavelength of 518 μm when excited at 396 μm; these settings were used for all measurements. Prior to each set of measurements, the spectrophotometer was adjusted to read a fluorescence intensity of 30% (instrument sensitivity set at 4) when a 0.1N H₂SO₄ solution containing 30 μg/ml quinine sulfate in a standard 1-cm cuvet was excited at a wavelength of 350 μm, and the fluorescence read at 446 μm. Excitation and emission slits were held at 7 (slit width of 0.91 mm) for both instrument standardization and measurement of the hydroxybenzpyrene. A standard curve plotting concentration of 3-hydroxybenz(a)pyrene (courtesy of Dr. H. V. Gelboin, Bethesda, Md.) versus percentage of fluorescence was constructed, and was linear throughout the range of concentrations obtained in these experiments.

Enzyme activity was expressed in units, with each unit equivalent to the formation of 100 μg hydroxylated metabolite/min reaction time.

RESULTS

The results of benzpyrene hydroxylase assays on the lungs of 95 control hamsters and 95 hamsters exposed to various concentrations of ozone are summarized in Table 1. All data were evaluated by several statistical tests, two of which are presented in the table, and described in the table footnotes.

The data presented demonstrate a decrease in measured activity of benzpyrene hydroxylase of 33% in animals exposed to 1,470 μg/cu m ozone and of 67% in animals exposed to both 5,880 μg/cu m and 19,600 μg/cu m. A few measurements were made of animals exposed to ozone at levels greater than 19,600 μg/cu m, but no decrease greater than 70% was observed.

Because of the relatively low levels of enzyme activity in normal hamster lung, a group of animals was treated 2 days prior to ozone exposure with an i.p. inoculation of 10 mg BaP, suspended in 2 ml sesame oil. After the initial stimulation, these animals were treated the same as nonstimulated animals. Pretreatment with BaP resulted in a 70-fold increase in control lung enzyme levels. The results of ozone exposure in these hamsters are summarized in Table 2.

In both the BaP-stimulated and nonstimulated animals, liver enzyme levels were measured for all exposure levels and room air. No exposed group was statistically different from the control levels of 36.7 ± 8.0 units/mg, wet weight, in normal hamsters and 231 ± 41.3 units/mg, wet weight, in pretreated hamsters.

As the ozone exposure level is increased, the amount of pulmonary reaction, especially edema formation, is also increased. Since the enzyme values are expressed in terms of units of activity/mg, wet weight, it is important to determine any diluent effects that edema formation might have.

Chart 1 is a plot comparing the ratio of exposed animal and control animal lung wet weights to the ratio of enzyme activities in these 2 groups. If the decrease in enzyme activity in exposed lungs were due only to dilution, the 2 curves would be identical. Assuming no difference in the lung dry weights of the exposed and control groups, the difference between the curves can only be attributed to the action of ozone. A study

<table>
<thead>
<tr>
<th>3-hr ozone treatment</th>
<th>No. of animals</th>
<th>Benzpyrene hydroxylase activity[a]</th>
<th>Statistical evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room air control</td>
<td>95</td>
<td>0.09 ± 0.002</td>
<td></td>
</tr>
<tr>
<td>1,470 μg/cu m (0.75 ppm)</td>
<td>25</td>
<td>0.06 ± 0.004</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>5,880 μg/cu m (3 ppm)</td>
<td>40</td>
<td>0.03 ± 0.003</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>19,600 μg/cu m (10 ppm)</td>
<td>30</td>
<td>0.03 ± 0.003</td>
<td>p &lt; 0.05</td>
</tr>
</tbody>
</table>

[a] Mean value ± S.E. Values are expressed as units of enzyme activity/mg, wet weight, of tissue assayed.

[b] A multiple comparison test whereby each treatment mean is tested for significance against the control mean.

[c] A multiple range test evaluating possible differences between all paired comparisons. Any two treatments connected by a vertical line are not significantly different at p = 0.05. All other paired comparisons are significantly different.

Effect of Ozone on Benzpyrene Hydroxylase

JUNE 1971
Michael S. Palmer, Donald H. Swanson, and David L. Coffin

**Table 2**

Benzpyrene hydroxylase activity in benzpyrene-stimulated hamster lungs

For explanatory footnotes, see Table 1.

<table>
<thead>
<tr>
<th>3-hr ozone treatment</th>
<th>No. of animals</th>
<th>Benzpyrene hydroxylase activity</th>
<th>Dunnett’s test</th>
<th>Duncan’s test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room air control</td>
<td>32</td>
<td>6.6 ± 0.4</td>
<td>p &lt; 0.05</td>
<td></td>
</tr>
<tr>
<td>1,470 μg/cu m (0.75 ppm)</td>
<td>12</td>
<td>4.3 ± 0.7</td>
<td>p &lt; 0.05</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>5,880 μg/cu m (3 ppm)</td>
<td>20</td>
<td>1.8 ± 0.6</td>
<td>p &lt; 0.05</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>19,600 μg/cu m (10 ppm)</td>
<td>8</td>
<td>2.0 ± 0.9</td>
<td>p &lt; 0.05</td>
<td>p &lt; 0.05</td>
</tr>
</tbody>
</table>

\^[a] The lack of a statistically significant difference between the 19,600 and 1,470 μg/cu m groups is thought to be due to the small number of animals examined in the former group. For this comparison, p < 0.1.

and, further, that increasing the exposure level to 19,600 μg/cu m (10 ppm) does not significantly increase this inhibition.

The benzpyrene hydroxylase enzyme system is present in most animal organs and is produced in increased amounts by organ exposure to BaP or other polyaromatic hydrocarbons. These experiments show that the degree of inactivation of pulmonary enzyme by ozone is independent of the actual amount of enzyme present at the time of exposure. The results present the possibility that inhalation of ozone might depress even stimulated enzyme levels enough to prolong the residence time of BaP and other carcinogenic hydrocarbons in the lungs. Clearance studies should give some idea of the extent of this prolonged residence, but any decreased BaP clearance in ozone-exposed animals could be caused by factors such as impaired macrophage function (8), as well as by decreased enzyme activity.

Since the main purpose of these experiments was to demonstrate whether or not there is an ozone effect, a single dose was used rather than chronic exposure. Actually, ozone levels in ambient air are not constant but are a series of peaks related, in part, to intensity of sunshine and amount of automobile exhaust. The average peak concentrations in urban atmospheres are somewhat lower than the 1470 μg/cu m (0.75 ppm) used in this study, although peak concentrations as high as 1780 μg/cu m (0.9 ppm) have been recorded (1).

Little controlled research has been done to evaluate the cocarcinogenic potential of various pollutant gases. In one study, however, Laskin et al. (16) have demonstrated a possible synergistic relationship between inhaled sulfur dioxide and BaP in rats, the combination of the two producing 5 carcinomas in 21 exposed animals. The effect of sulfur dioxide on enzyme activity has yet to be established, but work on this problem is currently under way.

Now that the direct toxicity of ozone on benzpyrene hydroxylase has been established, experiments must be conducted to evaluate the carcinogenic potential of this gas with BaP. Such experiments are currently underway in this laboratory, along with work that will elucidate the role of macrophage activity in the clearance of inhaled carcinogenic particles. Only when these studies are satisfactorily completed will we be able to evaluate properly the role of ozone in the pathogenesis of pulmonary cancer.

---

![Chart 1. Effect of pulmonary edema on lung benzpyrene hydroxylase activity in ozone-exposed hamsters.](chart1.png)
REFERENCES

Effect of Ozone on Benzpyrene Hydroxylase Activity in the Syrian Golden Hamster

Michael S. Palmer, Donald H. Swanson and David L. Coffin


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/31/6/730

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.