Inhibition of Induction of Benzpyrene Hydroxylase by Nickel Carbonyl

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SUMMARY

Exposure of rats to nickel carbonyl by inhalation (0.2 mg Ni/liter of air/15 min) or intravenous injection (2 mg Ni/100 gm) was found to inhibit the induction of benzpyrene hydroxylase in lung and liver. Phenothiazine induction and fluorometric measurements of benzpyrene hydroxylase were performed by the methods of Wattenberg and co-workers (31, 32). Benzpyrene hydroxylase activities in lungs and livers of control rats were 9.5 (S.D. ± 4.3) and 295 (S.D. ± 123) units, respectively. Benzpyrene hydroxylase activities began to diminish 3 hours after administration of nickel carbonyl, reached a minimum at 2 to 3 days, and remained diminished up to 1 week. For example, 52 hours after injection of nickel carbonyl, benzpyrene hydroxylase activities in lungs and livers were 1.1 ± 1.0 (P < 0.01) and 113 ± 24 (P < 0.01) units, respectively. The inhibition of benzpyrene hydroxylase activity was apparently mediated either by diminished synthesis or increased catabolism of the enzyme, inasmuch as nickel carbonyl did not have any direct effect upon benzpyrene hydroxylase activity, in vivo or in vitro. It is suggested that by inhibiting benzpyrene hydroxylation, nickel carbonyl may prolong the tissue retention of 3,4-benzpyrene and thereby promote carcinogenesis.

INTRODUCTION

In an attempt to identify the early biochemical changes which are associated with nickel carcinogenesis, the subcellular reactions of nickel have been investigated following exposure of rats to nickel carbonyl (27-29). In previous investigations, increased concentrations of nickel have been found in the microsomal and supernatant fractions of lungs and livers from exposed rats (28). Within the supernatant fraction, nickel has been shown to be bound predominantly to RNA and protein constituents (29). Increased binding of nickel to RNA from lung and liver has been associated with alterations in the thermal denaturation curves of the ribonucleic acids (27). These observations have led us to undertake the present investigation of the effects of nickel carbonyl upon the regulation of enzyme synthesis. In this study, measurements have been made of the induction of benzpyrene hydroxylase in lungs and livers of normal rats and of rats exposed to nickel carbonyl.

Benzpyrene hydroxylase is a microsomal enzyme which detoxifies 3,4-benzpyrene (18). The enzyme is present in lung, liver, intestine, and adrenals (9, 30, 32) and requires reduced triphosphopyridine nucleotide (TPNH) and oxygen for activity. Synthesis of benzpyrene hydroxylase is stimulated by administration of the substrate, 3,4-benzpyrene (2, 20), and by 3-methylcholanthrene (4, 8, 9, 20), dimethylbenzanthracene (4, 5), and phenothiazine (31). Loeb and Gelboin (19) have shown that such compounds induce benzpyrene hydroxylase by increasing the production of messenger RNA. Induction of benzpyrene hydroxylase has been reported in isolated, perfused rat livers (14). Benzpyrene hydroxylase is not induced by cortisone (31), and induction of the enzyme is not affected by adrenalectomy (19). Several investigations have demonstrated that puromycin (4, 8, 9), actinomycin D (4, 8, 9, 14), and ethionine (3, 4) inhibit the induction of benzpyrene hydroxylase. As will be described in the present paper, inhalation or intravenous injection of nickel carbonyl also inhibits the induction of benzpyrene hydroxylase.

MATERIALS AND METHODS

Induction and measurement of benzpyrene hydroxylase were performed by the methods of Wattenberg and co-workers (31, 32), with minor modifications. Female rats of the Sprague-Dawley strain (140–170 gm) were maintained on Purina laboratory chow. For induction of benzpyrene hydroxylase, 15 mg of recrystallized phenothiazine (dissolved in 1 ml of sesame oil) were administered by gastric intubation. Exactly 48 hours later, the rats were sacrificed by guillotine. The lungs and livers were homogenized (1.25%, w/v) in cold isotonic KCl using a Servall homogenizer at 15,000 rpm for 90 seconds.

Two ml of homogenate were pipetted into each of 4 Erlenmeyer flasks (10-ml capacity) containing 50 μg of 3,4-benzpyrene dissolved in 0.1 ml of acetone. One ml of reaction mixture was added to 2 of the flasks in order to initiate the reaction. The reaction mixture contained TPNH (1 mg); reduced diphosphopyridine nucleotide (DPNH) (0.05 mg); niacinamide (0.06 m); KCl (0.05 m); and phosphate buffer (pH 7.4, 0.05 m). One ml of blank mixture was added to the 2 remaining flasks. The blank mixture was identical to the reaction mixture, except that TPNH and DPNH were omitted. The flasks were incubated aerobically with constant shaking at 37°C in a covered water bath. After 20 min of incubation, the flasks were transferred to an ice bath and 3 ml of cold acetone were added in order to stop the reaction. Nine ml of n-hexane were added and the flasks were stopped and shaken. Aliquots of the organic phase (2 ml for lung; 1 ml for liver) were transferred to centrifuge tubes containing 1 ml NaOH (5 ml for lung, 10 ml for liver). The tubes were...
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stopped and the contents were mixed with a vortex rotatory mixer. The tubes were centrifuged at 2000 rpm for 1 min to separate the aqueous and organic phases.

Fluorescence of the aqueous extract was measured with a Farrand photoelectric fluorometer, Model A, equipped with a primary filter with maximum transmission at 400 m, and a secondary interference filter with maximum transmission at 522 m (half band width = 5 m). Fluorometry was performed within 5 min after obtaining the aqueous extracts. Readings of the blank samples were subtracted from those of the reaction samples, to correct for slight background fluorescence. A quinine sulfate solution (0.3 µg/ml of 0.1 N H₂SO₄) was used as a daily reference standard for adjustment of the fluorometer. A specimen of 8-hydroxy-3,4-benzpyrene was used for preparation of a calibration curve, which was found to be linear throughout the range of measurements. Accuracy of the analytic procedure was verified by assays of tissue extracts which were prepared and standardized by Dr. L. W. Wattenberg and Mr. J. L. Leong. Benzpyrene hydroxylase activity was computed in units which are equivalent to the formation of 0.1 µg of 8-hydroxy-3,4-benzpyrene/mg of wet tissue/min.

Administration of nickel carbonyl by inhalation and by intravenous injection was performed as previously described (10, 15). The LD₅₀ dosage of nickel carbonyl by inhalation was 0.20 mg Ni/liter of air/15 min. The LD₅₀ dosage by injection into a tail vein using a microsyringe was 2.2 mg Ni/100 gm (10). In rats that succumbed following inhalation or intravenous injections of nickel carbonyl in LD₅₀ dosage, death usually occurred on the 4th or 5th day (10). LD₅₀ dosages of nickel carbonyl were employed throughout the present investigation, except for an experiment in which nickel carbonyl was injected intravenously in sublethal dosage (0.4 mg Ni/100 gm). In an experiment to test the antidotal effect of a chelating agent, sodium diethylthiocarbamate trihydrate was administered to rats intraperitoneally in dosage of 10 mg/100 gm, 2 hours prior to intravenous injection of nickel carbonyl.

RESULTS

Measurements of benzpyrene hydroxylase activities in lung and liver homogenates from control rats and from rats exposed to nickel carbonyl are listed in Table 1 and are illustrated in Charts 1-4. In these charts, the activities of benzpyrene hydroxylase in lungs and livers from control rats are shown by the stippled columns and are contrasted with the activities in rats which received nickel carbonyl, as shown by the diagonally hatched columns. The heights of the columns represent the mean activities of the enzyme, and the vertical lines indicate the standard

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Phenothiazine induction*</th>
<th>Hours between induction and Ni(CO)₄</th>
<th>Hours between Ni(CO)₄ and sacrifice</th>
<th>No. of rats</th>
<th>Lung hydroxylase activity (units)</th>
<th>Liver hydroxylase activity (units)</th>
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<tr>
<td>Control</td>
<td>No</td>
<td>16</td>
<td>0.06 ± 0.52</td>
<td>0.2-1.9</td>
<td>18.5 ± 7.6</td>
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<td>2</td>
<td>Yes</td>
<td>25</td>
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<td>4.6-17.4</td>
<td>295 ± 123</td>
<td>120-520</td>
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<td>Ni(CO)₄ inhalation</td>
<td>3</td>
<td>No</td>
<td>+0.5</td>
<td>1.07 ± 0.37</td>
<td>0.5-1.8</td>
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<td>4</td>
<td>Yes</td>
<td>+47.5</td>
<td>10.2 ± 3.0</td>
<td>8.0-12.0</td>
<td>N.S.</td>
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<tr>
<td></td>
<td>5</td>
<td>Yes</td>
<td>-4</td>
<td>1.5 ± 1.1</td>
<td>0.2-3.3</td>
<td>&lt;0.01</td>
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<td>Intravenous Ni(CO)₄</td>
<td>6</td>
<td>No</td>
<td>+52</td>
<td>12</td>
<td>0.27 ± 0.29</td>
<td>0.0-0.8</td>
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<td></td>
<td>7</td>
<td>Yes</td>
<td>+47</td>
<td>7.6 ± 4.2</td>
<td>3.2-16.0</td>
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<tr>
<td></td>
<td>8</td>
<td>Yes</td>
<td>+45</td>
<td>10.5 ± 2.1</td>
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<td>9</td>
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<td>-28</td>
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<td>&lt;0.05</td>
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<td>-4</td>
<td>8.7 ± 4.4</td>
<td>2.3-13.7</td>
<td>&lt;0.01</td>
</tr>
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</table>

* Phenothiazine in sesame oil, 15 mg per rat by gastric intubation, 48 hours before sacrifice.
* Probability based upon null hypothesis for differences between values for experimental (nickel carbonyl) groups and corresponding control groups.
* Nickel carbonyl, 0.2 mg Ni/liter of air (80 ppm), inhalation for 15 minutes.
* NiCARB, not significant.
* Nickel carbonyl, 2 mg Ni/100 gm, intravenous injection.
* Number of surviving rats in these experimental groups. The starting complement of rats in these experimental groups was 12.
* Nickel carbonyl, 0.4 mg Ni/100 gm, intravenous injection.
* Sodium diethylthiocarbamate trihydrate, 10 mg/100 gm, intraperitoneal injection, 54 hours before sacrifice.
* P value versus Group 10.
deviations. The experimental groups contained an average of 11 rats (range = 4–25). In all experimental groups which received phenothiazine in order to induce synthesis of benzpyrene hydroxylase, the administration of phenothiazine by gastric intubation was performed 48 hours before sacrifice.

As shown in Chart 1, inhalation of nickel carbonyl a half hour before sacrifice did not significantly affect the activities of benzpyrene hydroxylase in lungs or livers of noninduced rats or of rats which had received phenothiazine induction. When nickel carbonyl was inhaled 4 hours before phenothiazine induction (52 hours before sacrifice), the activities of benzpyrene hydroxylase were significantly inhibited in homogenates of lungs and livers (P < 0.01). The mean activity of benzpyrene hydroxylase in lungs of exposed rats was 16% of that in the controls, and the mean activity of benzpyrene hydroxylase in livers of exposed rats was 58% of that in the controls.

Similar inhibitions of benzpyrene hydroxylase activities were observed following intravenous injection of nickel carbonyl (Chart 2). When nickel carbonyl was injected intravenously 4 hours before phenothiazine induction (52 hours before sacrifice), benzpyrene hydroxylase activities were diminished in both lung and liver (P < 0.01). The mean activity of benzpyrene hydroxylase in lungs of exposed rats was 12% of that in the controls, and the mean activity of benzpyrene hydroxylase in livers of exposed rats was 38% of that in the controls. The inhibition of benzpyrene hydroxylase was partially prevented by intraperitoneal adminis-
Inhibition of Benzpyrene Hydroxylase Induction

As shown in Chart 3, maximum inhibition of pulmonary benzpyrene hydroxylase activity was observed when nickel carbonyl was injected 4 hours before phenothiazine induction (52 hours before sacrifice). Significant inhibition was also observed when nickel carbonyl was injected up to 45 hours after phenothiazine induction (3 hours before sacrifice). Measurements of benzpyrene hydroxylase in rats which received nickel carbonyl 3 and 5 days before phenothiazine induction (5 and 7 days before sacrifice) were influenced by the fact that approximately half of the rats had died before the end of the experiment. Nonetheless, significant inhibition (P < 0.05) of pulmonary benzpyrene hydroxylase was observed when nickel was administered as long as 5 days before phenothiazine (7 days before sacrifice).

As indicated in Chart 4, maximum inhibition of hepatic benzpyrene hydroxylase was observed when nickel carbonyl was injected 28 hours before phenothiazine induction. Significant inhibition (P < 0.01) was also observed when nickel carbonyl was injected up to 45 hours after phenothiazine induction (3 hours before sacrifice), and as long as 3 days before phenothiazine (5 days before sacrifice).

Lung and liver homogenates were prepared from rats which had received intravenous nickel carbonyl in LD50 dosage 52 hours before sacrifice. These homogenates were boiled for 15 min, and 1-ml aliquots of filtrates of the boiled preparations were added to 1-ml aliquots of homogenates of lungs and livers from phenothiazine-induced rats. The filtrates of the boiled homogenates from nickel-treated rats did not inhibit benzpyrene hydroxylase activity. Similarly, no inhibition of benzpyrene hydroxylase activity was observed when nickel carbonyl (1 mg Ni) was added.
in vitro to homogenates of lungs and livers from phenothiazine-induced rats.

Intravenous injection of nickel carbonyl in sublethal dosage of 0.4 mg Ni/100 gm, 4 hours before phenothiazine administration, produced significant diminutions in the activities of benzpyrene hydroxylase in lung and liver (Table 1). The mean activity of pulmonary benzpyrene hydroxylase in exposed rats was 34% of that in the controls (P < 0.01), and the mean activity of hepatic benzpyrene hydroxylase in exposed rats was 70% of that in the controls (P < 0.05). None of these rats developed clinical signs of nickel poisoning. Pathologic examinations of rats sacrificed 4 days after intravenous injection of nickel carbonyl in dosage of 0.4 mg Ni/100 gm did not reveal gross or microscopic abnormalities. The hypertrophy and hyperplasia of alveolar lining cells which are characteristically observed following lethal exposures to nickel carbonyl (10) did not develop at the dosage level of 0.4 mg Ni/100 gm.

DISCUSSION

The present study has demonstrated that the administration of nickel carbonyl to rats by inhalation or intravenous injection inhibits phenothiazine induction of benzpyrene hydroxylase in lung and liver. The inhibition of benzpyrene hydroxylase induction appears to be mediated by either diminished synthesis or increased catabolism of benzpyrene hydroxylase, inasmuch as nickel carbonyl did not have an inhibitory action upon the enzyme in vitro. Moreover, the nickel which was present in filtrates of boiled homogenates of lungs and livers of exposed rats did not inhibit benzpyrene hydroxylase activity. No inhibition of benzpyrene hydroxylase in vitro was observed when rats were sacrificed immediately after inhalation or injection of nickel carbonyl. The inhibitory effect of nickel upon the induction of benzpyrene hydroxylase was more pronounced in the lung than in the liver. This observation is consistent with our previous finding that the lung is the target tissue for nickel carbonyl, regardless of its route of administration (10).

The effect of nickel carbonyl upon the induction of benzpyrene hydroxylase cannot be explained on the basis of a nonspecific inflammatory reaction, since the inhibition of enzyme induction was present at a dosage level of nickel carbonyl (0.4 mg Ni/100 gm) which did not produce any clinical or pathologic reactions. In addition, the maximum effect of nickel carbonyl upon pulmonary benzpyrene hydroxylase was observed when nickel carbonyl was injected 4 hours before phenothiazine induction (2 days before sacrifice). In contrast, the acute pathologic reactions to nickel carbonyl did not reach peak severity until the 4th or 5th day after nickel exposure (10).

Studies of the effect of nickel carbonyl upon induction of hepatic tryptophan pyrrolase and of aminopyrine N-demethylase are currently being undertaken in an attempt to elucidate the mechanisms of action of nickel carbonyl. Electron microscopic studies are being directed to the effects of nickel carbonyl upon the sequence of ultrastructural alterations which occur during the induction of benzpyrene hydroxylase. Measurements are also being made of the retention of administered 3,4-benzpyrene in lungs and livers of control rats and of rats that have received nickel carbonyl. The results of these studies will be reported in subsequent papers.

Several investigators have studied the enzymatic metabolism of 3,4-benzpyrene as a prototype of protective systems for the detoxification of carcinogenic polycyclic hydrocarbons (6, 11, 12, 13, 17). Through the action of benzpyrene hydroxylase, 3,4-benzpyrene is metabolized to noncarcinogenic, hydroxy derivatives (3, 6, 13, 15, 31). Carcinogenesis with polycyclic aromatic hydrocarbons, such as 3,4-benzpyrene, requires the persistence of nonmetabolized polycyclic hydrocarbons at the sites of administration or selective localization (1, 21, 22-26). Kotin and Falk (16) have suggested that the irregular incidence of carcinomas in populations exposed to polycyclic hydrocarbons may be explained by individual variations in the ability to detoxify the carcinogens. Falk et al. (7) have recently demonstrated that safrole, a hepatic carcinogen, interferes with hepatic detoxification and elimination of 3,4-benzpyrene. The present investigation indicates that nickel carbonyl, a pulmonary carcinogen, can interfere with an enzymatic mechanism for the detoxification of 3,4-benzpyrene. Interference in the detoxification mechanism may possibly promote pulmonary carcinogenesis by prolonging the retention of polycyclic hydrocarbons in the lung. Since 3,4-benzpyrene and nickel are both constituents of tobacco smoke and polluted urban air, the metabolic relationships of these compounds may be of importance in the pathogenesis of pulmonary neoplasia.

It should be emphasized that the effect of nickel carbonyl on the detoxification of 3,4-benzpyrene does not contradict the previous reports of a direct carcinogenic action of nickel carbonyl. The literature which pertains to the experimental production of cancers by nickel has recently been reviewed by Hackett and Sunderman (10).

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