Nickel Carbonyl Inhibition of Cortisone Induction of Hepatic Tryptophan Pyrrolase

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SUMMARY

Administration of nickel carbonyl to rats by intravenous injection (2 mg Ni/100 gm body weight) inhibited the induction of hepatic tryptophan pyrrolase by cortisone but not by tryptophan. Cortisone induction was impaired on the day after administration of nickel carbonyl. It reached a minimum at 2 days and remained diminished for 5 days. The average activity of hepatic tryptophan pyrrolase in cortisone-treated control rats was 36 (S.D. ±7) units, compared with an average activity of 20 ± 4 units in similar rats that received nickel carbonyl 2 days before sacrifice (P < 0.01). Cortisone induction of tryptophan pyrrolase was inhibited by nickel carbonyl in adrenalectomized rats as well as in normal rats. Nickel carbonyl did not inhibit tryptophan pyrrolase activity in vitro. The finding that nickel carbonyl inhibited induction of tryptophan pyrrolase by cortisone but not by tryptophan suggests that the toxic effect of nickel carbonyl on enzyme induction is mediated either by diminished synthesis or denaturation of messenger RNA.

INTRODUCTION

The literature which pertains to the experimental production of cancers by nickel has recently been summarized by Hackett and Sunderman (22). In the previous study in our series of investigations of nickel carcinogenesis, exposure of rats to nickel carbonyl by inhalation or intravenous injection was shown to inhibit phenothiazine induction of benzpyrene hydroxylase in lung and liver (47). It was concluded that the inhibition of enzyme induction by nickel carbonyl might result from either diminished synthesis or increased catabolism of the enzyme. The present study was undertaken in an attempt to identify which of these mechanisms is responsible for the action of nickel carbonyl upon enzyme induction. Hepatic tryptophan pyrrolase (EC 1.13.1.12) was selected as the experimental system, since measurements of tryptophan pyrrolase activity after induction with cortisone and with tryptophan furnish a method for differentiating the sites of action of inhibitors of enzyme induction (15, 34, 41).

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MATERIALS AND METHODS

Induction and measurement of hepatic tryptophan pyrrolase were performed by the methods of Pitot and associates (6, 36) with several minor modifications. Male rats of the Sprague-Dawley (Dublin Farms) strain (3 months old; 180-220 gm) were maintained on Purina laboratory chow and water ad libitum. Adrenalectomized male rats of the Sprague-Dawley (Charles River Farms) strain (1 month old; 70-90 gm) were maintained for at least 7 days after adrenalectomy on Purina laboratory chow and 0.9% (w/v) NaCl ad libitum. For substrate induction, the rats were given intraperitoneal injections at zero time and at 3 hr of 4 ml of an 0.25 M suspension of L-tryptophan in 0.9% (w/v) NaCl, and were sacrificed 6 hr after the initial injection. For cortisone induction, the rats were given intraperitoneal injections at zero time and at 3 hr of 10 mg of cortisone acetate in 1 ml of 0.9% (w/v) NaCl and were sacrificed 6 hr after the initial injection. The rats were killed by guillotine and exsanguinated in a cold room at 10°C. Portions of the livers were taken for histologic examination to verify the viability of the tissue. In adrenalectomized rats, the perirenal tissues were dissected to verify the completeness of adrenalectomy.

Liver homogenates (20% w/v in cold 0.154 M KCI) were prepared with a Teflon-glass tissue grinder, as described by Eaves et al. (9). Assays of tryptophan pyrrolase activity were performed in duplicate. Three-tenths ml of homogenates was added to duplicate 10-ml Erlenmeyer flasks containing: 0.3 ml of 0.03 M L-tryptophan; 1 ml of Tris buffer (pH 7.5; 0.2 M); 1 ml of 0.03 M sodium ascorbate (freshly prepared); and 20 µl of 0.025% (w/v) hematin (freshly prepared). Water was added to a final volume of 4 ml. The flasks were flushed with O2 and were incubated aerobically with constant shaking at 37°C in a water bath. At 15 min and at 75 min after the start of incubation, 1-ml portions of the reaction mixtures were removed and added to centrifuge tubes containing 1 ml of 8% (w/v) trichloroacetic acid. The tubes were centrifuged at 2000 rpm for 10 min to remove proteins. A portion (0.25-1.0 ml) of each supernatant was transferred to a spectrophotometer cuvet and diluted to 2 ml with 4% (w/v) trichloroacetic acid. To "blank" and "standard" cuvets were added 2 ml of 4% (w/v) trichloroacetic acid. One ml of distilled water was added to each of the sample cuvets and to the "blank" cuvet. One ml of kynurenine standard (0.02 µmole/ml) was added to the "standard" cuvet. Two-tenths ml of 0.25% (w/v) sodium nitrite (freshly prepared) was added to all of the cuvets, and the contents were mixed. Exactly 2 min later, 0.2 ml of 10% (w/v) ammonium sulfamate was added, and the contents were mixed.

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Exactly 2 min later, 0.2 ml of 0.25% (w/v) N-1-naphthylethylenediamine dihydrochloride was added, and the contents were mixed. After 30 min, the absorbance of the cuvets were measured at 550 mµ. Under the reaction conditions, the calibration curve was linear throughout the range of measurements, and enzymatic activity was linear throughout the period of incubation. The absorbances which were obtained with samples taken from the enzymatic reaction mixture after 15 min of incubation were subtracted from those obtained with samples taken after 75 min of incubation. Tryptophan pyrrolase activity was computed in units which are equivalent to the formation of 1 µmole of kynurenine per gm of wet tissue per hr.

Nickel carbonyl (2 mg Ni/100 gm body weight) was administered by intravenous injection into a tail vein with a microsyringe, as previously described (22). In nonadrenalectomized rats that died following this LD60 dosage (22) of nickel carbonyl, the deaths usually occurred on the 4th or 5th days. All of the adrenalectomized rats that received nickel carbonyl died by the 2nd day after the injection. Rats in control groups were given tail-vein injections of 5 µl of 0.9% (w/v) NaCl 24 hr before sacrifice, in order to provide sham manipulations similar to the nickel carbonyl injections.

RESULTS

Measurements of tryptophan pyrrolase activities in liver homogenates from nonadrenalectomized rats are listed in Table 1. Corresponding measurements in liver homogenates from adrenalectomized rats are listed in Table 2.

Injection of nickel carbonyl 24 or 48 hr prior to sacrifice resulted in slightly increased activity of tryptophan pyrrolase in livers from noninduced, nonadrenalectomized rats ($P < 0.01$). This effect is attributed to adrenal stimulation, since no increase was observed when the experiment was repeated with adrenalectomized rats.

Injection of nickel carbonyl 48 hr prior to sacrifice of nonadrenalectomized rats resulted in inhibition of cortisone induction of tryptophan pyrrolase ($P < 0.01$), but did not affect tryptophan induction. Cortisone induction of tryptophan pyrrolase was significantly diminished on the day after administration of nickel carbonyl; reached a minimum of 56% of the control value on the 2nd day, and remained diminished on the 3rd and 5th days. In surviving rats, cortisone induction of tryptophan pyrrolase was normal by the 7th day after injection of nickel carbonyl.

Injection of nickel carbonyl 24 hr prior to sacrifice of adrenalectomized rats resulted in inhibition of cortisone induction ($P < 0.01$), but did not affect tryptophan induction. The degree of inhibition observed at 24 hr in adrenalectomized rats (72% of control value) was similar to that observed at 24 hr in nonadrenalectomized rats (74% of control value).

No inhibition of tryptophan pyrrolase activity was observed when nickel carbonyl (0.5 mg Ni) was added in vitro to liver homogenates from cortisone-induced rats. Liver homogenates from rats which had received nickel carbonyl 48 hr before sacrifice were boiled for 15 min, and 0.3-ml portions of filtrates of the boiled preparations were added to 0.3-ml portions of liver homogenates from cortisone-induced rats. The filtrates of boiled homogenates from nickel-treated rats did not inhibit tryptophan pyrrolase activity in liver homogenates from cortisone-induced rats.

### TABLE 1

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Induction</th>
<th>Hours between Ni(CO)₄ and sacrifice</th>
<th>No. of rats</th>
<th>Tryptophan pyrrolase activity (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean ± S.D. ± Range ± P *</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>None</td>
<td>24</td>
<td>21</td>
<td>8.5 ± 2.7 ± 3.3-12.2 ± &lt;0.01</td>
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<tr>
<td>2</td>
<td>Tryptophan</td>
<td>48</td>
<td>10</td>
<td>7.5 ± 2.6 ± 5.4-14.1 ± 0.01</td>
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<tr>
<td>3</td>
<td>Cortisone</td>
<td>48</td>
<td>16</td>
<td>18.8 ± 4.7 ± 13-33 ± N.S.</td>
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<tr>
<td>Nickel carbonyl</td>
<td>None</td>
<td>120</td>
<td>9</td>
<td>31.1 ± 5.9 ± 24-38 ± &lt;0.05</td>
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<tr>
<td>8</td>
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<td></td>
</tr>
</tbody>
</table>

* Sprague-Dawley strain rats, male, 3 months old, 180-220 gm.

* Probability based upon null hypothesis for differences between values for experimental (nickel carbonyl) groups and corresponding control groups, computed by Student's "t" test.

* Tryptophan (0.25 ml suspension in 0.9% NaCl); 4 ml injected i.p. at 6 hr and 3 hr prior to sacrifice.

* Cortisone acetate (10 mg/ml of 0.9% NaCl); 1 ml injected i.p. at 6 hr and 3 hr prior to sacrifice.

* Nickel carbonyl (2 mg Ni/100 gm body wt.), intravenous injection.

* N.S., not significant.

* Number of surviving rats in these experimental groups. The starting complement of rats in these experimental groups was 12.
DISCUSSION

Tryptophan pyrrolase is an enzyme in the 105,000 X g supernatant fraction of rat liver homogenates (10) which catalyzes the aerobic oxidation of tryptophan to formylkynurenine (25). The enzyme has also been found in liver homogenates from mouse (42). For the enzyme (3, 21, 24), on the other hand, tryptophan reacts with a half-life of 2-2.5 hr (3, 13, 24). In comparison with the control values for adrenalectomized (1 month old) and nonadrenalectomized (3-month-old) rats are consistent with the findings of Correli et al. (8) regarding the changes in tryptophan pyrrolase induction in rats aged 1-12 months. 

Several studies have shown that the rates of synthesis and degradation of tryptophan pyrrolase in rat liver are very rapid, with a half-life of 2-2.5 hr (3, 13, 24). In comparison with the rapid turnover of the enzyme, the messenger RNA templates for tryptophan pyrrolase are relatively stable, with an estimated lifetime of more than 2 weeks (33, 37). Investigations in vivo (7, 17), in isolated, perfused rat livers (44), and in mouse liver slices (14) have established that independent mechanisms are involved in the induction of tryptophan pyrrolase by cortisone and by tryptophan. Cortisone acts at the level of genetic transcription, increasing the synthesis of messenger RNA templates for the enzyme (3, 21, 24). On the other hand, tryptophan reacts directly with the enzyme to decrease its rate of degradation (41, 42).

As previously mentioned, measurements of tryptophan pyrrolase activity after induction with cortisone and tryptophan provide an experimental system for differentiating the sites of action of inhibitors of enzyme induction. The most thoroughly studied examples are the effects of puromycin and actinomycin D. Puromycin acts at the ribosomal level to inhibit protein synthesis (3, 19), producing a prompt diminution in the concentration of tryptophan pyrrolase (13, 31). Owing to the short half-life of the enzyme, puromycin inhibition of enzyme synthesis effectively prevents induction by both cortisone and tryptophan (13, 15, 19, 31, 32). In contrast, actinomycin D inhibits DNA-dependent synthesis of messenger RNA (39, 40, 49) and thereby blocks cortisone induction of tryptophan pyrrolase without altering tryptophan induction (4, 13, 17, 18, 24, 30). Ionizing radiation has recently been reported to inhibit tryptophan induction of tryptophan pyrrolase without affecting cortisone induction (20). The mechanism whereby irradiation inhibits tryptophan induction of the enzyme has not yet been elucidated.

The present study has demonstrated that nickel carbonyl inhibits cortisone induction of tryptophan pyrrolase without inhibiting tryptophan induction. Differential inhibition of cortisone- and substrate induction of tryptophan pyrrolase has also been observed in the regenerating liver of partially hepatectomized rats (5, 43), coincident with the rapid increase in DNA synthesis which occurs 24 hr after partial hepatectomy (49). Similar findings of inhibition of cortisone induction but not of substrate induction of tryptophan pyrrolase have recently been reported for aflatoxin B1, a hepatocarcinogenic mycotoxin (12), and for bacterial endotoxin from Salmonella typhimurium (4). Differential inhibition of cortisone and tryptophan induction has not been observed with carbon tetrachloride (29). The hepatic injury produced by carbon tetrachloride decreases the basal level of tryptophan pyrrolase but does not damage the enzyme induction systems (29).

Under the conditions of the present investigation, nickel carbonyl did not have a direct action upon tryptophan pyrrolase

## TABLE 2

**Effect of Nickel Carbonyl upon Hepatic Tryptophan Pyrrolase in Adrenalectomized Rats**

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Induction</th>
<th>Hours between Ni(CO)4 and sacrifice</th>
<th>No. of rats</th>
<th>Tryptophan pyrrolase activity (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>None</td>
<td></td>
<td>10</td>
<td>2.0 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>Tryptophan</td>
<td></td>
<td>7</td>
<td>5.6 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>Cortisone</td>
<td></td>
<td>27</td>
<td>6.5 ± 2.1</td>
</tr>
<tr>
<td>Nickel carbonyl</td>
<td>None</td>
<td>24</td>
<td>10</td>
<td>1.7 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Tryptophan</td>
<td>24</td>
<td>7</td>
<td>5.8 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>Cortisone</td>
<td>24</td>
<td>9</td>
<td>4.7 ± 1.0</td>
</tr>
</tbody>
</table>

* Sprague-Dawley strain rats, male, 1 month old, 70-90 gm.
* Probability based upon null hypothesis for differences between values for experimental (nickel carbonyl) and control groups, computed by Student's "t" test.
* L-Tryptophan (0.25 M suspension in 0.9% NaCl); 4 ml injected i.p. at 6 hr and 3 hr prior to sacrifice.
* Cortisone acetate (10 mg/ml of 0.9% NaCl); 1 ml injected i.p. at 6 hr and 3 hr prior to sacrifice.
* Nickel carbonyl (2 mg Ni/100 gm body wt); intravenous injection.

** Legend:**
- Ni(CO)4: Nickel carbonyl
- Tryptophan: Tryptophan
- Cortisone: Cortisone
- S.D.: Standard deviation
- Range: Range of values

- N.S., not significant.
- * Probability based upon null hypothesis for differences between values for experimental (nickel carbonyl) and control groups, computed by Student's "t" test.
- ** Number of surviving rats in these experimental groups. The starting complement of rats in these experimental groups was 18.

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per se, inasmuch as: (a) nickel carbonyl did not inhibit the enzyme in vitro; (b) nickel was present in filtrates of boiled homogenates of liver from nickel-treated rats did not inhibit the enzyme; and (c) nickel carbonyl inhibition was observed only in cortisone-induced rats, and not in noninduced or tryptophan-induced rats. The inhibition of cortisone induction of tryptophan pyrrolylase by nickel carbonyl cannot be attributed to liver regeneration following hepatic necrosis, since histologic examination of the rat livers revealed only mild pathologic changes, and these changes did not develop until 4-5 days after injection of nickel carbonyl. The pathologic findings in liver of rats sacrificed 5 days following administration of nickel carbonyl included mild centrilobular congestion and diminished intensity of periodic acid-Schiff staining.

Based upon the results of the present investigation, it appears probable that the toxic effect of nickel carbonyl on enzyme induction is mediated by a metabolic block at the level of messenger RNA, without disturbance of protein synthesis from pre-existing ribosomal templates. The metabolic block could result either from diminished synthesis or denaturation of RNA. It is speculated that the action of nickel carbonyl might be produced by: (a) inhibition of 5'-nucleotidase (45); (b) competitive antagonism of RNA, without disturbance of protein synthesis from pre-existing polymerase (11); or (c) denaturation of messenger RNA (46), so that it cannot be incorporated as a ribosomal template. Studies of the subcellular metabolism of \( ^{57}\text{Ni} \text{(CO)}_4 \) and measurements of the incorporation of orotic acid-\(^{14}\text{C} \) into liver messenger RNA are currently being undertaken in our laboratory in an attempt to elucidate the site of the toxic action of nickel carbonyl. Investigations are also being directed to the possible inhibitory effects of other nickel compounds and of other metallic carboxyls upon enzyme induction.

It should be emphasized that there is no evidence that the present finding of inhibition of enzyme induction by nickel carbonyl is directly related to nickel carcinogenesis. However, Pitot and Heidelberger have proposed a theoretic model of metabolic regulatory circuits that might explain the perpetuation of metabolic changes brought about by the binding of a carcinogen to messenger RNA or to endogenous repressor proteins (35). The observation that nickel carbonyl is a potent inhibitor of enzyme induction is compatible with the Pitot-Heidelberger hypothesis of chemical carcinogenesis, in which the earliest stage of carcinogenesis involves interruption of the regulatory circuits for enzyme synthesis (35).

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