Effect of Polycyclic Aromatic Compounds and Phorbol Esters on Ornithine Decarboxylase and Aryl Hydrocarbon Hydroxylase Activities in Mouse Liver

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ABSTRACT

Single i.p. injections of 3-methylcholanthrene (MC; 50 mg/kg) administered to inbred C57BL/6 mice or 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; 100 µg/kg) to DBA/2 mice gave an increase in the hepatic activities of ornithine decarboxylase (ODC) and aryl hydrocarbon hydroxylase (AHH) with peaks occurring by 12 and 48 hr, respectively. A single i.p. dose of the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA; 100 µg/kg) enhanced the activity of ODC about 70-fold within 12 hr in C57BL/6 mice and 18-fold within 24 hr in DBA/2 mice without affecting AHH activity markedly. 4-O-Methyl-12-O-tetradecanoylphorbol-13-acetate (100 µg/kg) raised ODC activity to about 25% of the TPA-treated value in C57BL/6 mice; in DBA/2 mice, TPA and 4-O-methyl-12-O-tetradecanoylphorbol-13-acetate induced ODC activity to roughly the same level. Benzo(a)pyrene (50 mg/kg) failed to affect ODC and AHH activities significantly in either strain. The inducing effect of TPA on ODC activity was potentiated by a simultaneous administration of MC to C57BL/6 mice; combined TPA and TCDD to DBA/2 mice exerted an additive effect on hepatic ODC activity. Difluoromethylornithine administered i.p. effectively inhibited the induction of ODC activity elicited by TPA, MC, or TCDD either alone or in various combinations but did not interfere with AHH induction.

These data indicate that different regulatory factors are involved in the ODC induction process elicited by TPA and polycyclic aromatic compounds and that MC and TCDD may induce ODC activity by different mechanisms. The results also confirm our earlier findings in rat skin and cells in culture which suggest that the ODC and AHH induction processes can occur independently of each other. Additionally, there is a strain-related difference in sensitivity with regard to ODC-inducing activity of TPA in the livers of C57BL/6 and DBA/2 mice.

INTRODUCTION

ODC (EC 4.1.1.17) is the rate-limiting enzyme in the biosynthesis of the polyamines putrescine, spermidine, and spermine, which play an essential role in both normal and neoplastic cellular growth (reviewed in Refs. 12 and 14). Induction of ODC activity by phorbol esters, e.g., TPA, has proved to be an early marker of tumor-promoting action in mouse epidermal cells in vivo (29) and in vitro (41). Systemically administered TPA has been shown to induce the activity of ODC in tissues other than skin, e.g., in the liver of mice (16) and in the liver and lung of rats (39), suggesting that TPA may be able to serve as a promoter in these and perhaps other tissues (1, 10, 39).

AHH (EC 1.14.14.2), one of the substrate-inducible microsomal monooxygenase systems, is involved in the conversion of polycyclic aromatic compounds to potent carcinogenic intermediates which interact with cellular DNA (reviewed in Refs. 9 and 32). The murine Ah locus regulates the induction of the numerous xenobiotic-metabolizing enzyme activities in the liver by polycyclic aromatic compounds, such as MC or TCDD (38). The induction of AHH occurs in MC-treated C57BL/6 mice and other genetically responsive inbred strains and is always much lower in MC-treated DBA/2 mice and other genetically nonresponsive strains (26). However, a large dose of TCDD (60 or 100 µg/kg) will elicit a response in AHH activity also in DBA/2 mice (20). Several animal studies have demonstrated an association between genetically mediated high AHH inducibility and susceptibility to carcinogenesis by certain polycyclic compounds (18-22).

Chemical carcinogens, including polycyclic aromatic compounds, enhance the activity of ODC in the liver (3, 5, 24), and it has been postulated that the induction of ODC activity represents an early event in the sequence of subcellular changes occurring between the time when the inducer first enters the cell and the time when the induced microsomal monooxygenase activities reach their maximal extent (28). Prolonged elevation of ODC activity may also be necessary for hepatocarcinogenesis (30, 31).

We have previously shown that p.o. administration of 1,3-diamino-2-propanol, an indirect inhibitor of ODC, to rats and mice inhibited hepatic monooxygenase activities (35). The fact that 1,3-diamino-2-propanol is a nonspecific inhibitor of ODC with other pharmacological effects makes clear-cut interpretations difficult. We therefore tested the hypothesis that the induction of AHH is mediated via ODC induction by utilizing the newly discovered, virtual nontoxic, highly selective inhibitor of ODC, namely, DFMO (25). The present results show that, although MC and TCDD stimulate both ODC and AHH activities in mouse liver, the 2 processes occur independently, since AHH is normally activated under conditions where ODC activity is markedly reduced.

MATERIALS AND METHODS

Materials

MC was purchased from Fluka AG (Buchs, Switzerland); B(e)P from Aldrich Chemical Co. (Milwaukee, Wis.); TPA and 4-O-methyl-TPA from Consolidated Midland Corp. (Brewster, N. Y.); DL-L(14C)ornithine (specific activity, 56 mCi/mmol), CO2mMet, and phase-combining system...
were from Amersham International, Ltd. (Amersham, United Kingdom). DFMO was kindly given to us by Dr. D. J. Wilkins (Centre de Recherche Merrell International, Strasbourg, France), and TCDD was given by Dr. D. W. Nebert (NIH, Bethesda, Md.). All other reagents were of the highest grade commercially available.

**Treatment of the Animals**

Male C57BL/6 and DBA/2 mice (5 weeks old; about 20 g) received standard rodent chow and tap water *ad libitum* and were kept in a room with controlled temperature and humidity and a photoperiod from 6 a.m. to 6 p.m. MC or B(e)P in corn oil were injected i.p. as a single dose (50 mg/kg), and TCDD (100 μg/kg) dissolved in dioxane and TPA or 4-methyl-TPA (100 μg/kg in acetone or ethanol) were injected i.p. with a Hamilton precision syringe (Hamilton Bonaduz AG, Bonaduz, Switzerland); the amount of these solvents injected was always less than 10 μl. DFMO (500 mg/kg) was administered i.p. in 0.9% NaCl solution. The animals were killed by cervical dislocation between 8 a.m. and 9 a.m., and the livers were rapidly removed, cleansed of blood, and subsequently homogenized with ice-cold 100 mM Tris-HCl buffer (pH 7.2) containing 5 mM dithiothreitol, 1 mM EDTA, 0.2 mM pyridoxal phosphate, and 0.1 mM L-ornithine. Four volumes of buffer were used per g of liver.

**Analytical Methods**

**AHH Assay.** The method used was the fluorimetric procedure of Nebert and Gelboin (27). The incubation mixtures contained 62.5 μmol potassium phosphate buffer (pH 7.4), 2.5 μmol MgCl2, 50 μmol KCl, 1.5 μmol glucose-6-phosphate, 0.06 μmol NADP, 5 units glucose-6-phosphate dehydrogenase, 80 nmol benzo(a)pyrene, and 25 μl of the liver homogenate in a volume of 1.0 ml. The incubations were carried out for 15 min at 37°C. The reaction was stopped by addition of 1.0 ml of acetone, and the mixture was shaken with 3.25 ml of n-hexane for 10 min. A 1.0-ml aliquot of the organic layer was transferred into 3.0 ml of 1 N NaOH, and the fluorescence was measured at 390 nm excitation and 520 nm emission. Duplicate samples were always assayed. AHH specific activity is expressed in nmol 3-hydroxybenzo(a)-pyrene (or equivalents) formed per g, wet weight, of tissue per min.

**ODC Assay.** The liver homogenates were centrifuged for 30 min at 30,000 × g, and 100-μl aliquots of the supernatant fraction were assayed in duplicate for ODC activity by determining the amount of 14CO2 released from 0.5 μCi of DL-[1-14C]ornithine essentially as described by Beaven et al. (2). A 1 × 1 × 1-cm piece of filter paper saturated with 100 μl of ethylene glycol:CO2/mixture (1:1, v/v) was placed in the bottom of a 20-ml screw-capped liquid scintillation vial. The incubation mixtures were placed in 1.5-ml Eppendorf tubes which were left open and placed inside the liquid scintillation vials, which were then closed tightly. The buffer used in the assay was the same as the homogenization buffer. The vials were incubated by shaking in a water bath at 37°C for 30 min, after which 250 μl of 50% trichloroacetic acid were added to the incubation mixture and the incubation was continued for an additional 30 min. At the end of the second incubation, the Eppendorf tubes were removed, 7 ml of phase-combining system:xylene (2:1, v/v) were added to each scintillation vial, and the radioactivity was determined. The specific activity of ODC is expressed in nmol CO2 formed per mg protein per 30 min.

**RESULTS**

**Time Course of Hepatic ODC Induction.** As shown in Chart 1, a single i.p. injection of MC (50 mg/kg) into C57BL/6 mice or TCDD (100 μg/kg) into DBA/2 mice resulted in rapid stimulation of hepatic ODC activity, the peak occurring 12 hr after injection in both strains (6- and 10-fold induction, respectively). The activity of ODC declined only slowly in the MC-treated C57BL/6 mice over a 48-hr period, whereas the activity in the TCDD-treated DBA/2 mice declined to nearly the basal level by 24 hr and then increased again slightly. A single i.p. dose of TPA (100 μg/kg) led to a marked transient induction of ODC activity in the C57BL/6 mice. The peak of activity (about 70-fold greater than in the vehicle-treated controls) occurred at 12 hr and declined to the control level by 48 hr. The response of the DBA/2 mice to TPA was much less pronounced, the activity being enhanced about 18-fold, with peak at 24 hr.

More than 10-fold differences in the maximally induced ODC activities were found among the individual TPA-treated mice. This variability, which is beyond the magnitude of the experimental error in the ODC assay, is most probably due to the extremely short half-life of the enzyme (10 to 20 min) together with day-to-day variations.

**Time Course of Hepatic AHH Activity.** Hepatic AHH activity increased significantly after a single dose of either MC or TCDD (Chart 2) and was maximally elevated at 48 hr in both C57BL/6 and DBA/2 mice (approximately 5- and 6-fold induction, respectively). Administration of TPA was totally ineffective in raising the AHH activity in either strain.

**Dose-Response Relationships.** The effect of varying single doses of MC, TCDD, and TPA on hepatic ODC and AHH activities is shown in Table 1. The maximal induction of ODC activity was attained with a 50-mg/kg dose of MC to the C57BL/6 mice and a 500-μg/kg dose of TCDD to DBA/2 mice. The dose response for TPA in the C57BL/6 mice differs markedly from that obtained with the DBA/2 mice. The main difference was that the maximal response in ODC activity was...
obtained with TPA (100 μg/kg) in the C57BL/6 mice, the overtly toxic dose of 500 μg/kg produced no further increase in ODC activity in the C57BL/6 mice, whereas this toxic dose overtly toxic dose of 500 μg/kg produced no further increase in hepatic ODC activity; due to large variations among the

Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ODC activity (nmol/mg protein/30 min)</th>
<th>AHH activity (nmol/g tissue/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6 (control)</td>
<td>0.01 ± 0.01 a</td>
<td>13.53 ± 1.02</td>
</tr>
<tr>
<td>MC, 1 mg/kg</td>
<td>0.01 ± 0.01</td>
<td>32.77 ± 4.20</td>
</tr>
<tr>
<td>MC, 10 mg/kg</td>
<td>0.04 ± 0.01</td>
<td>38.46 ± 3.34</td>
</tr>
<tr>
<td>MC, 50 mg/kg</td>
<td>0.06 ± 0.02</td>
<td>41.69 ± 4.41</td>
</tr>
<tr>
<td>MC, 100 mg/kg</td>
<td>0.04 ± 0.01</td>
<td>55.51 ± 7.94</td>
</tr>
<tr>
<td>TPA, 1 μg/kg</td>
<td>0.01 ± 0.01</td>
<td>16.07 ± 1.41</td>
</tr>
<tr>
<td>TPA, 10 μg/kg</td>
<td>0.03 ± 0.02</td>
<td>13.87 ± 0.66</td>
</tr>
<tr>
<td>TPA, 100 μg/kg</td>
<td>0.72 ± 0.53</td>
<td>11.84 ± 3.67</td>
</tr>
<tr>
<td>TPA, 500 μg/kg</td>
<td>0.61 ± 0.39</td>
<td>8.53 ± 0.26</td>
</tr>
<tr>
<td>DBA/2 (control)</td>
<td>0.01 ± 0.01</td>
<td>14.81 ± 0.71</td>
</tr>
<tr>
<td>TCDD, 10 μg/kg</td>
<td>0.03 ± 0.01</td>
<td>21.66 ± 2.66</td>
</tr>
<tr>
<td>TCDD, 100 μg/kg</td>
<td>0.12 ± 0.05</td>
<td>36.96 ± 11.22</td>
</tr>
<tr>
<td>TCDD, 500 μg/kg</td>
<td>0.30 ± 0.12</td>
<td>19.58 ± 2.32</td>
</tr>
<tr>
<td>TPA, 1 μg/kg</td>
<td>0.02 ± 0.01</td>
<td>14.65 ± 2.10</td>
</tr>
<tr>
<td>TPA, 10 μg/kg</td>
<td>0.04 ± 0.01</td>
<td>16.36 ± 2.67</td>
</tr>
<tr>
<td>TPA, 100 μg/kg</td>
<td>0.12 ± 0.02</td>
<td>11.57 ± 2.24</td>
</tr>
<tr>
<td>TPA, 500 μg/kg</td>
<td>0.32 ± 0.17</td>
<td>8.86 ± 1.76</td>
</tr>
</tbody>
</table>

The mice were given a single i.p. injection of the compounds at the doses indicated and were killed 14 hr later for ODC and AHH determinations.

The animals were treated as in Table 2 except that MC was replaced with TCDD (100 μg/kg).
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groups, this difference was not statistically significant. The combination of TPA and TCDD elicited a 34-fold increase in ODC activity and a 1.7-fold increase in AHH activity. DFMO inhibited TCDD-induced ODC activity by 60%, but the corresponding AHH activity was not significantly affected. DFMO was much less effective in inhibiting ODC induction produced by the combination of TPA and and TCDD than it had been in inhibiting MC plus TPA-induced ODC activity in C57BL/6 mice. The reason for this is obscure.

Liver Histology. To determine the morphological response of the liver to the compounds used, 2 mice were necropsied 14 hr after injection of a single dose of MC (50 mg/kg), TCDD (100 μg/kg), and TPA (100 μg/kg), alone or in combinations, and liver slices were studied by light microscopy. No toxic tissue damage could be seen; however, slight variation in hepatocellular nucleus size and shape was observed in the treated animals.

DISCUSSION

The results showed that ODC activity in C57BL/6 and DBA/2 mouse livers is induced by the phorbol esters TPA and 4-O-methyl-TPA and the polycyclic compounds MC and TCDD, but not significantly by the weakly carcinogenic B(e)P. The response of C57BL/6 and DBA/2 mice to systemically adminis-tered TPA and 4-O-methyl-TPA was strikingly different; in the C57BL/6 mice, hepatic ODC activity was induced about 70-fold 14 hr after a single i.p. injection of TPA (100 μg/kg), whereas the nonpromoting 4-O-methyl-TPA increased the activity of ODC only 17-fold. The extent of ODC induction by TPA was much smaller in the DBA/2 strain, and TPA and 4-O-methyl-TPA increased ODC activity to roughly the same level. These findings suggest that the increase in hepatic ODC activity in the DBA/2 strain may correlate with the hyperplasogenic activity of the phorbol ester rather than with the tumor-promot- ing activity.

There is evidence for the existence of a cytosolic receptor for polycyclic aromatic compounds (11, 33). The Ah-regulatory gene encodes the cytosolic receptor capable of binding inducers of the polycyclic aromatic type. The inducer-receptor complex is translocated into the nucleus, resulting in transcription of specific mRNA’s into specific enzymes such as AHH (26). ODC induction occurs during various conditions under which gene activation is enhanced (14), including activation of the AHH structural gene (28). Interaction of tumor-promoting phorbol esters with specific receptors in the cell membrane may be one of the earliest events in triggering the biological responses produced by these compounds (8). Although the mechanism that brings on detectable increases in ODC activity remains obscure, it is conceivable that polycyclic compounds stimulate receptor systems involved in ODC induction, one possibility being the TPA receptor. However, the present finding that hepatic ODC activity is “superinduced” by combined TPA and MC in C57BL/6 mice suggests some other site of interaction. When the combination of TPA and TCDD was tested for its ODC-inducing effect in DBA/2 mice, the result was roughly additive, suggesting that the induction of ODC by TCDD may occur through different mechanisms than that by MC.

There are conflicting reports about the ability of monoxygenase inducers to influence carcinogenesis. Depending upon the experimental conditions and tissue studied, elevated monoxygenase activity may also lead to decreases rather than to increases in polycyclic hydrocarbon-induced neoplasia. For example, TCDD when administered i.p. or s.c. enhances the carcinogenic index in DBA/2 mice treated with s.c. MC (20). On the other hand, TCDD pretreatment inhibits the carcinogenicity of polycyclic hydrocarbons when they are applied as skin tumor initiators in Sencar mice (4) or CD-1 mice (7). Additionally, TCDD has been reported to reduce the amount of benzo(a)-pyrene metabolite/DNA adducts in the lungs of A/ HeJ mice, suggesting that TCDD should actually protect against polycyclic hydrocarbon-induced neoplasia (40).

Thus, the relationship between monoxygenase induction and carcinogenesis is not clear. It is apparent that the generation of reactive intermediates and their interaction(s) with critical subcellular targets is highly dependent upon the balance between toxification and detoxification factors, which may vary in different tissues, strains, and species. Moreover, different P-450-dependent isozymes in a tissue or cell type may catalyze divergent pathways of toxification and detoxifi-cation to a different degree (6, 15). The significance of tumor promotion-related events such as stimulation of tissue ODC activity in these differences remains to be elucidated.

A single i.p. dose of DFMO efficiently inhibited the enhance-ment in ODC activity induced by TPA or MC in C57BL/6 mice. A near-total inhibition of ODC induction did not affect the MC-induced AHH activity, however. Correspondingly, DFMO also inhibited TCDD-induced ODC activity in DBA/2 mice without interfering with the AHH induction process. This demonstrates that normal inducibility of hepatic AHH activity can occur without a preexisting peak in ODC activity. The results presented do not absolutely rule out the possibility that a specific polyamine is involved in the AHH induction process, since other studies have shown that, in some cases, the inhibition of ODC by DFMO results in an increase in the activity of S-adenosylmethionine decarboxylase and in the accumulation of spermine (17). We have shown previously that DFMO efficiently suppresses ODC activity in rat epidermis (36) and in cells of various origin in culture (34, 37) without interfering with poly-cyclic aromatic hydrocarbon-induced AHH activity. It thus seems likely that ODC induction per se is not an integral part of the sequence of cellular events caused by polycyclic aromatic compounds leading to AHH induction. Rather, it is a fortuitous event associated with a large number of pleiotypic responses which include growth and/or proliferation (14). Our early finding concerning the ability of 1,3-diamino-2-propanol to inhibit hepatic microsomal monoxygenase probably repre-sents a nonspecific inhibition not causally related to reduction of ODC activity (35).

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REFERENCES


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