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INDUCTION OF ARYL HYDROCARBON (BENZO[a]PYRENE) HYDROXYLASE AND 2-ACETYLAMINOFLUORENE *N*-HYDROXYLASE BY POLYCYCLIC HYDROCARBONS IN REGENERATING LIVER FROM INBRED STRAINS OF MICE*

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Abstract—The effect of partial hepatectomy (67–75 per cent excised) on aryl hydrocarbon (benzo[a]pyrene) hydroxylase and 2-acetylaminofluorene *N*-hydroxylase activities in genetically responsive C57BL/6N and nonresponsive DBA/2N inbred strains of mice was studied. Basal aryl hydrocarbon hydroxylase activity in C57BL/6N mice is reduced by 70 per cent within 12 hr but returns to control values between 36 and 48 hr after the operation and remains at that level thereafter. Similar, but less pronounced, changes are observed in the DBA/2N mouse. Treatment with 3-methylcholanthrene (80 mg kg^{-1} intraperitoneally) 1 hr after partial hepatectomy induces aryl hydrocarbon hydroxylase in C57BL/6N mice, by 48 hr, to the same extent as in mice with intact livers. In DBA/2N mice, even after partial hepatectomy, treatment with 3-methylcholanthrene has no effect on aryl hydrocarbon hydroxylase activity. The effect of partial hepatectomy and 3-methylcholanthrene treatment on 2-acetylaminofluorene *N*-hydroxylase activity in both strains of mice is similar to that observed on aryl hydrocarbon hydroxylase activity.

The aryl hydrocarbon hydroxylase in the liver of C57BL/6N mice is induced by 3-methylcholanthrene treatment, whereas this enzyme is not induced by 3-methylcholanthrene in the liver of DBA/2N mice. Although genetic differences in aryl hydrocarbon hydroxylase induction exist in cell culture from fetal mouse liver, cells from a strain normally nonresponsive to polycyclic hydrocarbons do exhibit some induction of aryl hydrocarbon hydroxylase activity [1]. Regenerating liver is similar to cell culture in that DNA is rapidly replicating and the hepatocytes are less differentiated. Thus, it is possible after partial hepatectomy that aryl hydrocarbon hydroxylase might become partially or completely inducible by polycyclic hydrocarbons in the liver of nonresponsive mice. This paper presents evidence that partial hepatectomy neither affects the maximum 3-methylcholanthrene-induced activities of aryl hydrocarbon hydroxylase and 2-acetylaminofluorene *N*-hydroxylase in the responsive C57BL/6N mice nor causes an increase in these enzyme activities after 3-methylcholanthrene treatment in the nonresponsive DBA/2N mice.

MATERIALS AND METHODS

The polycyclic hydrocarbons benzo[a]pyrene and 3-methylcholanthrene were purchased from Sigma Chemical Co. (St. Louis, MO) and J. T. Baker Chemical Co. (Phillipsburg, NJ), respectively; β-naphthoflavone was purchased from Aldrich Chemical Co. (Milwaukee, WI); 2-acetylaminofluorene from Eastman Kodak Co. (Rochester, NY); and NADPH and NADH from Sigma Chemical Co. [9-^{14}C]-2-acetylaminofluorene (10.5 mCi/m-mole), purchased from New England Nuclear (Boston, MA), was shown to be more than 99.9 per cent pure by thin-layer chromatography (chloroform–methanol, 97:3, v/v). Authentic *N*-hydroxy-2-acetylaminofluorene was generously given to us by Dr. Elizabeth Weisburger, National Cancer Institute. All other reagents were of the best commercial grade available. The inbred C57BL/6N and DBA/2N mice used in these studies were obtained from the National Institutes of Health Animal Supply.

Treatment of animals. The mice were kept on standard hardwood bedding in plastic cages and fed Wayne Lab-Blox chow *ad lib.* The environment in the animal room was controlled as previously described [2]. Mice of either sex [3], between 4 and 6 weeks of age at the time of operation, were used. For studies involving enzyme induction by the aromatic hydrocarbons 3-methylcholanthrene or β-naphthoflavone, 80 mg of the compound/kg of body weight, in corn oil, was administered intraperitoneally to each mouse at various time intervals before sacrifice; controls received an equivalent volume of corn oil alone.

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Partial hepatectomy. Partial hepatectomies (67–75 per cent removed) and sham operations were performed on C57BL/6N and DBA/2N mice under ether anesthesia between 9:00 a.m. and 12:00 noon, according to the method of Higgins and Anderson [4]. The partially hepatectomized mice were sacrificed at various time intervals after the operation for aryl hydrocarbon hydroxylase and *N*-hydroxylase assays. Regeneration of the liver during the 5 days after the operation was in accordance with the observations of Higgins and Anderson [4].

Preparation of microsomes. Animals were killed by decapitation. Immediately upon exsanguination the minced livers from individual mice were separately washed as free as possible from blood in ice-cold 250 mM potassium phosphate–KCl buffer, pH 7.25, and homogenized. The homogenate was centrifuged for 20 min at 9000 *g* and the supernatant fluid was carefully decanted and recentrifuged for 60 min at 105,000 *g*. The surface of the microsomal pellet was washed twice with the phosphate–KCl buffer, and the pellet was suspended in the same buffer prior to assay. Protein was determined according to the method of Lowry *et al.* [5].

Enzyme assays. The aryl hydrocarbon hydroxylase activity in liver microsomes was estimated as previously described by Nebert and Gielen [6]. One unit of aryl hydrocarbon hydroxylase activity is defined as the amount of enzyme catalyzing/min at 37° the formation of hydroxylated product causing fluorescence equivalent to that of 1 pmole of 3-hydroxybenzo[a]pyrene recrystallized standard.

The rate of *N*-hydroxylation of 2-acetylaminofluorene in liver microsomes was measured as previously described [7]. One unit of 2-acetylaminofluorene *N*-hydroxylase activity is defined as that amount of enzyme catalyzing/min at 37° the formation of 1 pmole *N*-hydroxy-2-acetylaminofluorene. The specific activities of both aryl hydrocarbon hydroxylase and 2-acetylaminofluorene *N*-hydroxylase are expressed in this report as units/mg of microsomal protein.

The amount of cytochrome P-450 in the microsomal fractions was estimated by the method of Omura and Sato [8].

Histology. Paraffin sections were prepared from livers obtained at different time intervals after partial hepatectomy, and stained with hematoxylin and eosin [9] for microscopic examination.

RESULTS

The aryl hydrocarbon hydroxylase activity in untreated mice was decreased for the first 48 hr after partial hepatectomy (Fig. 1). Whereas the decrease in the hydroxylase activity was pronounced in the C57BL/6N mice, only small and statistically not significant ($P > 0.05$) changes were observed in the DBA/2N mice. Studies of mono-oxygenase activities in the regenerating rat liver [10] have shown that both *p*-hydroxylation of aniline and *N*-demethylation of aminopyrine are decreased after partial hepatectomy and return to control levels at 60 and 180 hr respectively.

The changes in liver mono-oxygenase activity in the C57BL/6N mice observed after partial hepatectomy were paralleled by histological changes in the liver. During the first 36–48 hr the liver underwent reversible fatty infiltration, which was apparent both macroscopically and histologically. Within 2–3 days, however, both the gross and histologic appearance of the liver had returned to normal. Although mono-oxygenase activity in the liver of DBA/2N mice is not significantly decreased after partial hepatectomy, the histological changes were similar to those observed in C57BL/6N mice. Surgical mortality was 10–20 per cent, and mice survived successfully for at least 1 year after partial hepatectomy.

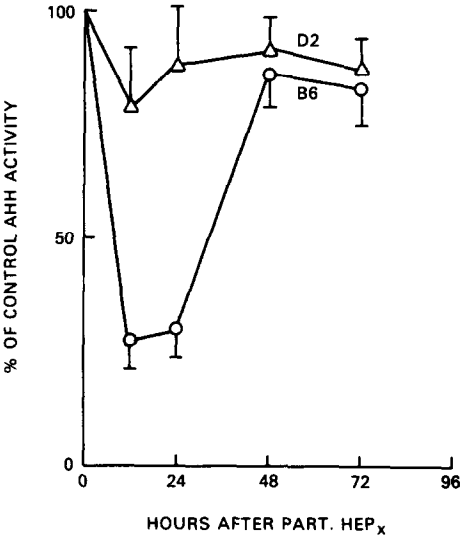

Fig. 1. Effect of partial hepatectomy on aryl hydrocarbon hydroxylase activity in C57BL/6N (B6) (○) and DBA/2N (D2) (△) mice. Sham operated mice were used as controls. Each point is a mean ± S.E.M. of six animals. The specific activity of aryl hydrocarbon hydroxylase in control animals was 320 ± 31 pmoles mg^{-1} min^{-1} for the C57BL/6N and 290 ± 37 pmoles mg^{-1} min^{-1} for the DBA/2N.

Induction of hepatic aryl hydrocarbon hydroxylase activity by polycyclic hydrocarbons such as 3-methylcholanthrene and β-naphthoflavone is found in some inbred strains of mice but not in others [6]. Figure 2 shows the effect of partial hepatectomy on 3-methylcholanthrene induction of aryl hydrocarbon hydroxylase in the genetically responsive C57BL/6N and the nonresponsive DBA/2N mouse. 3-Methylcholanthrene (80 mg kg^{-1}) was given intraperitoneally in corn oil 1 hr after surgery. In the responsive B6 mouse, induction of aryl hydrocarbon hydroxylase was detectable within 12 hr, and increased to a maximum at 48 hr, as compared with aryl hydrocarbon hydroxylase activity in the untreated operated control. A similar pattern of induction was observed in the C57BL/6N mouse, with maximum activity, at 48 hr, not differing significantly from that observed in the operated mice [3]. In the nonresponsive DBA/2N mouse, 3-methylcholanthrene treatment after partial hepatectomy did not alter the aryl hydrocarbon hydroxylase activity.

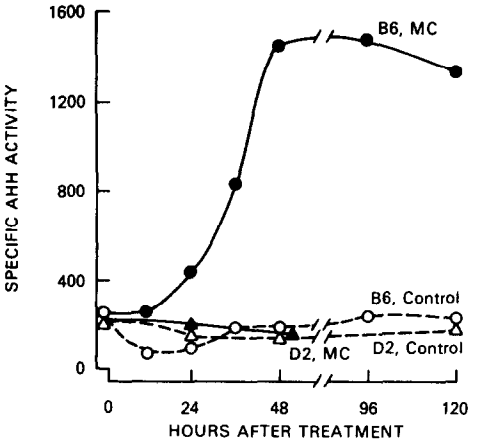

Fig. 2. Induction of aryl hydrocarbon hydroxylase in partially hepatectomized B6 and D2 mice with 3-methylcholanthrene (MC) (80 mg kg^{-1}, i.p.). Key: B6 control (O----O); B6 MC-treated (●——●); D2 control (Δ---Δ); and D2 MC-treated (▲——▲). Each point is a mean of eight animals.

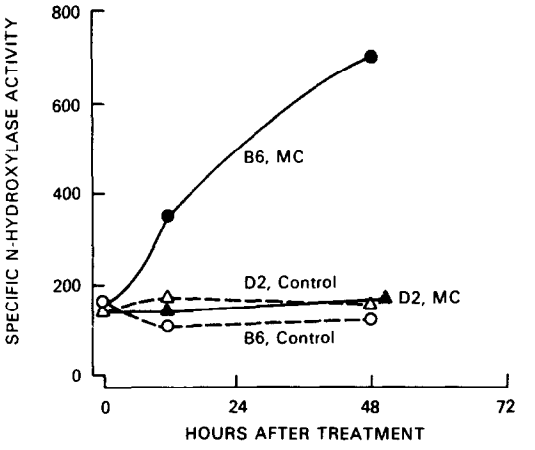

Fig. 3. Induction of 2-acetylaminofluorene *N*-hydroxylase in partially hepatectomized B6 and D2 mice with MC (80 mg kg^{-1}, i.p.). Key: B6 control (O---O); B6 MC-treated (●——●); D2 control (Δ---Δ); and D2 MC-treated (▲——▲). Each point is a mean of five animals.

The genetically mediated trait of aryl hydrocarbon hydroxylase inducibility by polycyclic hydrocarbons has recently been shown to be closely linked with the inducibility of the hepatic *N*-hydroxylation of 2-acetylaminofluorene in C57BL/6N and DBA/2N mice [2]. Figure 3 shows the effect of partial hepatectomy on the 3-methylcholanthrene induction of the *N*-hydroxylase in C57BL/6N and DBA/2N mice. After partial hepatectomy this genetic association was still apparent; the responsive C57BL/6N mouse showed induction of the *N*-hydroxylase after 3-methylcholanthrene treatment, whereas the *N*-hydroxylase in nonresponsive DBA/2N mouse was not induced. The magnitude of *N*-hydroxylase induction at 48 hr in the hepatectomized C57BL/6N mouse did not differ significantly from that found in unoperated animals. Pretreatment of the mice with β-naphthoflavone gave the same response in both C57BL/6N and DBA/2N with respect to aryl hydrocarbon hydroxylase and 2-acetylaminofluorene *N*-hydroxylase induction as did 3-methylcholanthrene.

Figure 4 shows the effect of partial hepatectomy on 3-methylcholanthrene-induced aryl hydrocarbon hydroxylase activity in C57BL/6N mice. The specific aryl hydrocarbon hydroxylase activity is plotted as a percentage of the maximum value found in unoperated 3-methylcholanthrene-treated animals as a function of the time between surgery and sacrifice of the animals. All animals received 80 mg kg^{-1} of 3-methylcholanthrene 48 hr prior to sacrifice. As the time between partial hepatectomy and sacrifice lengthened, induced aryl hydrocarbon hydroxylase activity increased, reaching 100 per cent of the unoperated control values at 48 hr. Although the absolute activity of aryl hydrocarbon hydroxylase was decreased over the first 24 hr after partial hepatectomy when compared with the corresponding nonoperated controls, fold inducibility (i.e. the ratio of control over induced enzyme activity) remained unaltered or even increased between 24 and 48 hr.

* A. R. Boobis, J. S. Felton and D. W. Nebert, manuscript in preparation.

It has previously been shown that administration of 3-methylcholanthrene or β-naphthoflavone to responsive inbred strains of mice (such as C57BL/6N, C3H or BALB/c) results in the formation of a microsomal CO-binding hemoprotein [6]. This hemoprotein, referred to as cytochrome P-448 or P_1-450, is characterized by a 2-nm shift to the blue in its CO-difference spectrum, as compared with the control value of 450 nm for control mice or 3-methylcholanthrene-treated nonresponsive mice. In addition, there is a close correlation between electrophoretic increases in this new hemoprotein and increases in aryl hydrocarbon hydroxylase activity after polycyclic aromatic hydrocarbon treatment of responsive mice.* Figure 5 shows that there was a selective induction of cytochrome P_1-450 after 3-methylcholanthrene treatment in partially hepatectomized C57BL/6N mice. The 2-nm shift to the blue in the CO-difference

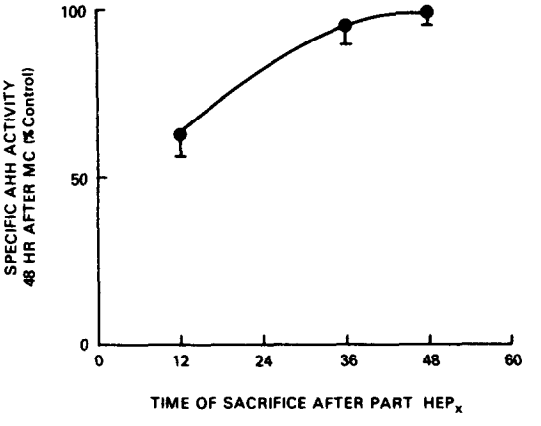

Fig. 4. Effect of partial hepatectomy on AHH activity in B6 mice after MC treatment. All animals received MC (80 mg kg^{-1}, i.p.) 48 hr prior to sacrifice. Thus, for example, animals at the 12-hr time point were operated on 36 hr after the injection of MC and sacrificed a further 12 hr later. Each point is a mean ± S.E.M. of five animals.

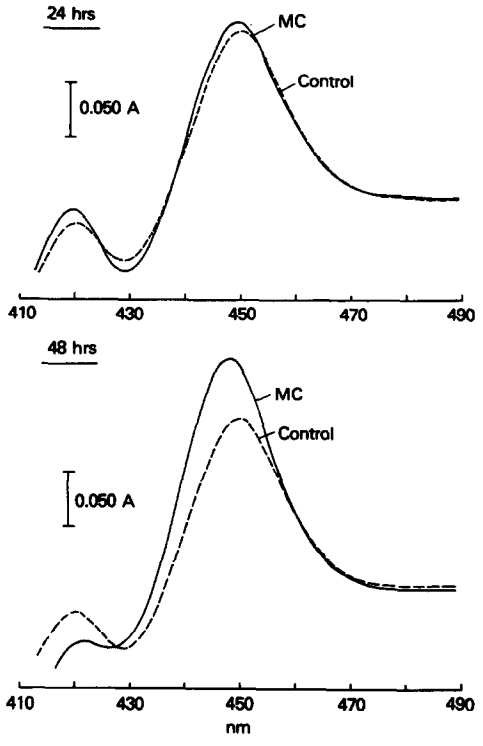

Fig. 5. Carbon monoxide difference spectra of hepatic microsomes 24 and 48 hr after partial hepatectomy in untreated and MC-treated B6 mice.

spectrum, as well as an increase of 40 per cent in total cytochrome P-450 content, was readily observed 48 hr after 3-methylcholanthrene treatment. Results similar to these were found in unoperated 3-methylcholanthrene-treated C57BL/6N mice.*

DISCUSSION

We have previously shown [2] that induction by polycyclic aromatic hydrocarbons of aryl hydrocarbon hydroxylase and 2-acetylaminofluorene *N*-hydroxylase activities is genetically linked in the inbred C57BL/6N and DBA/2N mice. After partial hepatectomy in the responsive C57BL/6N mouse, polycyclic aromatic hydrocarbons induce both aryl hydrocarbon hydroxylase and 2-acetylaminofluorene *N*-hydroxylase to the same extent as in unoperated C57BL/6N mice, with maximum induction occurring at 48 hr in all cases. This corresponds to the formation of cytochrome P_1-450 (or P-448) as determined spectrophotometrically. The aryl hydrocarbon hydroxylase and 2-acetylaminofluorene *N*-hydroxylase activities in the nonresponsive DBA/2N mouse are not induced by polycyclic aromatic hydrocarbons even after partial hepatectomy.

Both basal and polycyclic hydrocarbon-induced aryl hydrocarbon hydroxylase and *N*-hydroxylase activities are significantly decreased the first 24 hr after partial hepatectomy in the C57BL/6N mouse (Figs. 1 and 4). Similar changes have been observed in the rat after partial hepatectomy, and also in both rat and mouse after chemical liver injury [10–12]. The reason for the difference in the decrease of monooxygenase(s) activity after partial hepatectomy between the C57BL/6N and DBA/2N (Fig. 1) mice is not clear at this point, but it could possibly reflect some unknown strain difference in response to the partial hepatectomy. Despite the decrease after partial hepatectomy in basal aryl hydrocarbon hydroxylase activity in the responsive C57BL/6N mouse, 3-methylcholanthrene induction becomes apparent 12 hr after the injection of the polycyclic hydrocarbon (Fig. 2). Furthermore, the fold inducibility throughout the course of polycyclic hydrocarbon induction is the same or greater than in the unoperated C57BL/6N mouse.

It has previously been shown that aryl hydrocarbon hydroxylase in cell culture from DBA/2N mice, which are normally nonresponsive, is inducible by polycyclic hydrocarbons [1]. The possibility, therefore, exists that aryl hydrocarbon hydroxylase activity might be induced in the regenerating liver of the adult DBA/2N mouse. No induction of either aryl hydrocarbon hydroxylase or *N*-hydroxylase is observed in the regenerating liver from the DBA/2N mouse after the administration of polycyclic hydrocarbons, indicating that even during periods of rapid cell growth the genetic expression of these enzyme activities is similar to the intact liver.

There is increasing evidence to suggest that replicating cells are especially vulnerable to the action of carcinogens. The evidence stems mainly from work on skin carcinogens [13], but recently cell replication has been implicated in the initiation of carcinogenesis in liver [14]. The use of partially hepatectomized responsive C57BL/6N and nonresponsive DBA/2N mice as well as progeny from appropriate backcrosses and intercross [6, 15] could provide a valuable experimental model for evaluating the role of aryl hydrocarbon hydroxylase activity in chemically initiated liver cancer [16].

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