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SHORT COMMUNICATION

Genetic Expression of the Induction of Epoxide Hydrase and Aryl Hydrocarbon Hydroxylase Activities in the Mouse by Phenobarbital or 3-Methylcholanthrene

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SUMMARY

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Intraperitoneal administration of phenobarbital causes significant induction of hepatic epoxide hydrase activity in C57BL/6N, C3H/HeN, NZB/BLN, and NZW/BLN inbred strains of mice and not in DBA/2N, N:GP(SW), and AL/N strains, whereas a hepatic monooxygenase [aryl hydrocarbon (benzo[*a*]pyrene) hydroxylase] activity is stimulated 1.5–3-fold in all seven strains. Intraperitoneal administration of 3-methylcholanthrene has virtually no effect on the hydrase activity in any of the seven strains. The aromatic hydrocarbon increases the hydroxylase activity 2–6-fold in C57BL/6N, N:GP(SW), AL/N, and C3H/HeN mice but causes no significant rise in the hepatic enzyme activity in DBA/2N, NZB/BLN, and NZW/BLN mice. In appropriate crosses between C57BL/6N and DBA/2N, the expression of epoxide hydrase induction by phenobarbital appears so complex as to preclude any simple genetic analysis; the basal hydrase levels appear to be inherited additively.

The importance of aryl oxidations of drugs and polycyclic hydrocarbons to pharmacology, toxicology, and chemical carcinogenesis has recently been reviewed (1). The oxidative metabolism of these lipophilic compounds (Fig. 1) proceeds via the reactive arene oxide (epoxide) intermediates, which can isomerize to phenols, be converted enzymatically to *trans*-dihydrodiols or glutathione conjugates, or become covalently bound to cellular nucleic acids and proteins.

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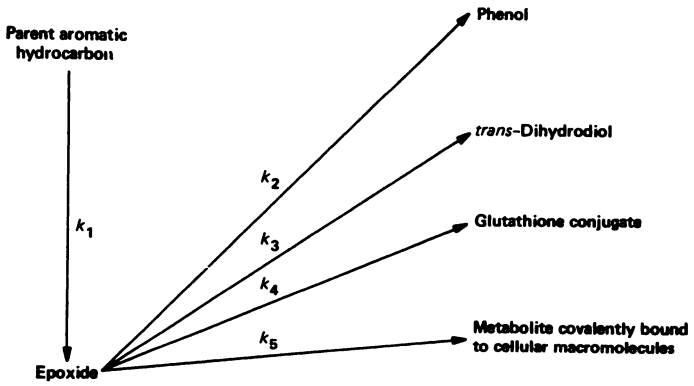

FIG. 1. *Possible metabolic pathways for formation and disappearance of oxides of aromatic hydrocarbons*

Both the hepatotoxicity (2–6) and carcinogenicity (7–10) of such xenobiotic compounds are dependent on metabolic activation and appear to be associated with the degree of reaction of the arene oxides with cellular macromolecules. Thus the cytotoxic or carcinogenic effects of a xenobiotic would presumably be decreased or prevented by increases in k_2, k_3, and k_4 or by decreases in k_1 (Fig. 1). Selective increases in epoxide hydrase (11) and glutathione-epoxide transferase (12) activities (reactions k_3 and k_4, respectively) and/or selective inhibition of aryl hydrocarbon hydroxylase activities (13) *in vivo* may accomplish this aim. There is, however, evidence (14) suggesting that the monooxygenase-epoxide hydrase systems in guinea pig liver microsomes are tightly coupled. This hypothesis is further supported by the finding (15) that monooxygenase and hydrase activities are not easily separated in partially purified preparations of soluble cytochrome P-450 and P-448 from rat liver microsomes. In bacteria the close linkage of functionally related genes commonly exists (16), most likely to offer the organism a greater opportunity to pass advantageous coordinated genetic information to its progeny. For example, the five coordinated enzymes involved in protoheme biosynthesis in *Staphylococcus aureus* are regulated by five tightly linked and cotransducible genes (17). The induction of aryl hydrocarbon hydroxylase activity by aromatic hydrocarbons, but not by phenobarbital, has been shown (18) to segregate as a single autosomal dominant trait in genetic crosses between certain inbred strains of mice. In this report we show that the genetic regulations of the phenobarbital- or 3-methylcholanthrene-inducible hydroxylase and epoxide hydrase activities are not closely associated, in spite of evidence that the enzyme proteins may be closely coupled in the microsomal membrane.

Various strains of mice from the National Institutes of Health Animal Supply were studied. The animals were always maintained in as rigidly a controlled environment (18) as possible. Unless otherwise indicated, sexually immature mice aged 3–6 weeks were used. Assays for epoxide hydrase (19) and aryl hydrocarbon hydroxylase (20) activities in liver microsomes were performed as described. The epoxide hydrase specific activity is expressed as nanomoles of [^{3}H]styrene glycol formed per minute per milligram of microsomal nitrogen; nitrogen content was determined by the micro-Kjeldahl method. One unit of aryl hydrocarbon hydroxylase activity is defined (20) as that amount of enzyme catalyzing, per minute at 37°, the formation of hydroxylated product causing fluorescence equivalent to that of 1 pmole of 3-hydroxybenzo[*a*]pyrene.[4] The hydroxylase specific activity is thus expressed in units per milligram of microsomal protein. 3-Methylcholanthrene treatment consisted of 80 mg/kg administered intraperitoneally 24 hr prior to death;

[4] With benzo[*a*]pyrene as the substrate *in vitro*, the "aryl hydrocarbon hydroxylase" assay measures the rate of formation of 3-hydroxybenzo[*a*]-pyrene; this phenol may be formed either by a direct hydroxylation or in a two-step process (reactions k_1 and k_2 of Fig. 1) (see ref. 1).

phenobarbital treatment consisted of 80 mg/kg administered intraperitoneally for 3 consecutive days, and the enzyme activities were assayed 24 hr following the third dose. Controls received the vehicles corn oil and 0.9% sodium chloride, respectively.

Figure 2 shows the expression of hepatic hydrase and hydroxylase activities among control, 3-methylcholanthrene-treated, and phenobarbital-treated mice of seven different strains. 3-Methylcholanthrene produced little increase in the hydrase activity in any of the strains examined, whereas it stimulated the hydroxylase activity in the inbred B6[5] and AL/N strains and in the randombred Swiss strain. As noted before (18), 3-methylcholanthrene did not induce the hydroxylase system in the D2, NZB/BLN, or NZW/BLN strains. On the other hand, phenobarbital stimulated hydroxylase activity about 1.5–3-fold in all seven strains of mice and had widely variable effects on the hydrase activity: 2–3-fold increases in hydrase activity were found in B6, C3H/HeN, NZB/BLN, and NZW/BLN inbred mice, yet barely detectable increases or no statistically significant differences were seen in the D2, N:GP(SW), and AL/N strains. For the B6 and D2 inbred strains, the development of the differences in epoxide hydrase induction by phenobarbital as a function of age is illustrated in Fig. 3. The basal hydrase levels in B6 or D2 mice and the enzyme activities in phenobarbital-treated D2 mice at 1 week of age were not statistically ($p > 0.05$) different from those in each respective group at the later ages. The phenobarbital-induced hydrase activity reached a maximum at about 3 weeks of age. The hydrase was not measurable, or was barely detectable and variable, in hepatic postmitochondrial supernatant fractions from 2-day-old and fetal mice. The enzyme activity in control B6 mice was always about 50% greater than that in control D2 mice.

The large individual variability in phenobarbital treated progeny of the B6 × D2 and B6D2 × B6D2 intercrosses and the B6D2 ×

[5] The abbreviations used are: B6, the C57BL/6N inbred mouse strain; D2, the DBA/2N inbred mouse strain.

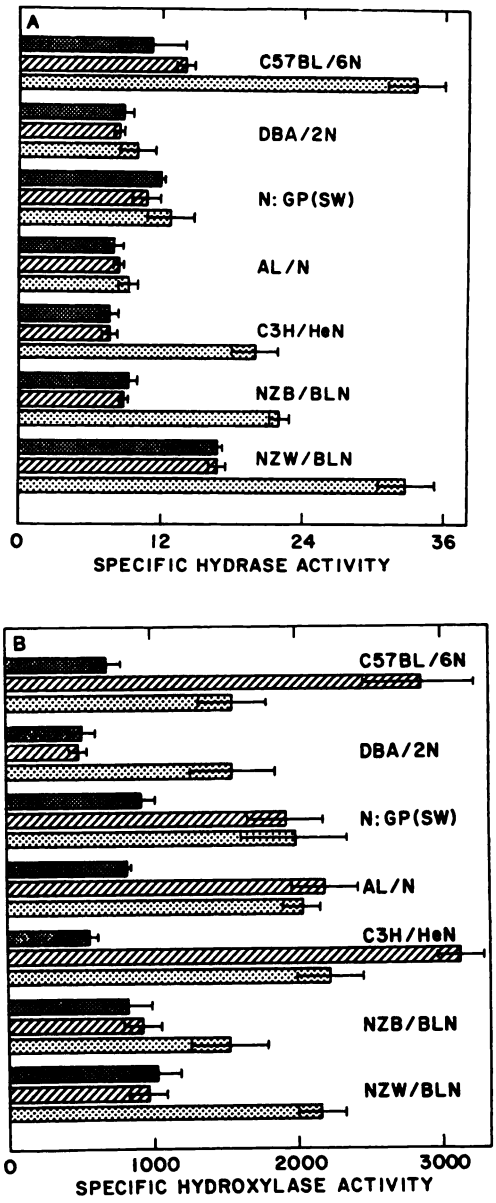

FIG. 2. *Specific activities of hepatic epoxide hydrase (A) and aryl hydrocarbon hydroxylase (B) from control, 3-methylcholanthrene-treated, and phenobarbital-treated mice of various strains*

The heavily stippled, striped, and lightly stippled bars represent the enzyme activities from control, 3-methylcholanthrene-treated, and phenobarbital-treated mice, respectively. The NIH general-purpose Swiss-Webster mouse N:GP (SW) is a randombred strain; the other six strains shown are inbred.

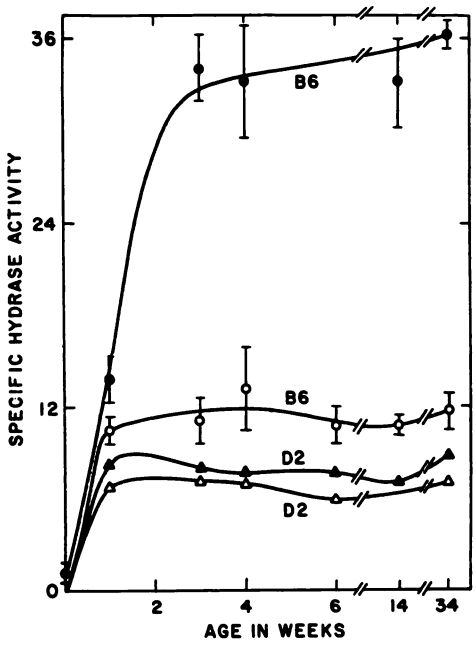

FIG. 3. *Ontogenetic expression of hepatic epoxide hydrase specific activity in control (open symbols) and phenobarbital-treated (solid symbols) C57BL/6N and DBA/2N mice*

Each point represents the mean of at least six mice; brackets denote standard deviations for each mean. Only males were examined at the 14- and 34-week time points.

backcross (Fig. 4) suggests that regulation of the hydrase induction by phenobarbital is too complex for any simple interpretation. This observation is in marked contrast to the single autosomal dominant expression found (18, 20–22) for aryl hydrocarbon hydroxylase induction by aromatic hydrocarbons in these two strains. Since each B6D2 F_1 or D2B6 F_1 animal has the same genetic constitution as every other B6D2 F_1 or D2B6 F_1 animal (except for differences on the X and Y chromosomes), the great variability of phenobarbital-inducible hydrase activity seen may be due partly to environmental causes. In other mammalian genetic studies, the induction of δ-aminolevulinic acid synthetase activity in mouse liver by 3,5-dicarbethoxy-1,4-dihydro-2,4,6-trimethylpyridine was also reported (23) to be complex. The induction of aldehyde dehydrogenase activity in rat liver cytosol by phenobarbital (24) is expressed additively. The distribution of the basal epoxide hydrase activity (Fig. 4B) suggests that the control enzyme is inherited additively.

In this report two mammalian enzyme activities, which are architecturally and functionally closely coupled entities of a membrane-bound system, are not organized as a type of eukaryotic operon, with respect to increases in these enzyme activities caused by typical microsomal enzyme "inducers." Since these enzyme activities are part of a

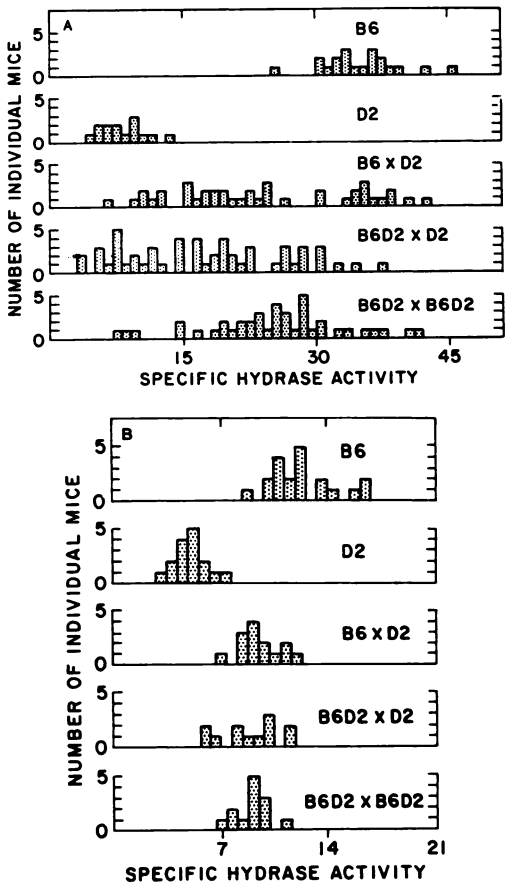

FIG. 4. *Genetic variance in hepatic epoxide hydrase specific activities among phenobarbital-treated (A) and control (B) C57BL/6N and DBA/2N mice and progeny of appropriate crosses*

Although the cross is shown as "B6 × D2," mice from both B6 × D2 and D2 × B6 crosses were examined, and no differences in the hydrase activity were found between B6D2 F_1 and D2B6 F_1 offspring. Likewise, no differences were seen among B6D2 × D2, D2B6 × D2, D2 × B6D2, and D2 × D2B6 offspring.

very complicated multicomponent enzyme system, these studies may not be relevant to the problem of regulation of enzymes by coordinate loci in eukaryotes. Indeed, there are no data at present suggesting linkage of genes for proteins catalyzing sequential metabolic reactions in mammalian cells. Finally, we suggest that well-defined, genetically mediated differences in the relative basal and inducible levels of the enzymes for xenobiotic metabolism in any given tissue (i.e., k_1, k_3, and k_4 of Fig. 1) may provide a valuable experimental approach to studies of cytotoxicity and carcinogenicity. In addition, it should be recognized that differences in the tissue pool size of glutathione, NADPH, and perhaps other factors will undoubtedly affect the steady-state levels of the reactive arene oxides at any particular time and dosage of the xenobiotic.

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