p-NITROPHENOL UDP GLUCURONYLTRANSFERASE AND EPOXIDE HYDRASE IN MICROSOMES FROM LIVER OF RATS FED 2-ACETYLAMINOFLUORENE AND BARBITAL

M. J. Griffin and K. K. Walston
Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma 73104

Liver microsomal *p*-nitrophenol UDP glucuronyltransferase and epoxide hydrase are induced significantly by feeding rats 2-acetylaminofluorene for two weeks. Barbital included in the diet results in a much smaller induction of both enzymes. Barbital does not affect the level of hydrase induced by 2-acetylaminofluorene when fed with the carcinogen, but it does reduce the transferase level to that induced by barbital alone when fed with 2-acetylaminofluorene. 2-Acetylamino-fluorene-induced hyperplastic nodules contain the highest levels of both enzymes observed. These results indicate that parent carcinogen detoxification pathways may be fully operational in preneoplastic tissue, possibly affording them selective advantage over their more normal surrounding tissue.

INTRODUCTION

Xenobiotic compounds as well as systemic unsaturated organic molecules are metabolized in liver to more-water-soluble compounds by a set of closely associated enzymes of the endoplasmic reticulum. The major reaction sequence is believed to involve oxidation of the unsaturated bond to an epoxide by one of a family of mixed function oxidases (P-450), hydrolysis of the epoxide by epoxide hydrase, and glucuronidation of the dihydrodiol or hydroxy derivative by UDP glucuronyltransferase. This is a mechanism which targets the original unsaturated molecule for excretion as the glucuronide conjugate.

2-Acetylaminofluorene (2-AAF) acts as a hepatocarcinogen when fed to male Holtzman rats at 0.05% of diet. The tumors which occur after 16 weeks of feeding are hepatomas (parenchymal cell cancers) and generally appear from one to three months after 2-AAF feeding is discontinued. At 18 weeks, well-defined nodules are apparent on the liver surface. These hyperplastic nodules show some characteristics of hepatoma and some of normal tissues, and are believed precursors of the tumor. Recently, it was discovered that a special protein in nodule microsomes was altered so that it was released upon *in vitro* incubation under conditions in which normal liver microsomes retained the protein (1, 2). This protein, which was detected immunologically, was called a preneoplastic (PN) antigen (1). It was purified (2) and identified as epoxide hydrase (3). Recent studies in our laboratory have shown that this PN antigen effect can be demonstrated initially only after two weeks of feeding 2-AAF, a time when epoxide hydrase is already fully induced (3). This in turn suggests an alteration in the organization of 2-AAF-metabolizing enzymes with respect to the membrane and, probably, to one another in the liver of rats fed 2-AAF for two weeks. Since UDP glucuronyltransferase activity is believed to be important in further modifying the dihydrodiols produced by epoxide hydrase, we have studied its acute and chronic regulation by 2-AAF, and contrasted this with its regulation by the classical inducer, barbital.

MATERIALS AND METHODS

Animals

White male Holtzman rats (150 to 170 g) were obtained from a breeding colony maintained at the Noble Foundation (Ardmore, Oklahoma). Rats were fed a basal diet (4) containing 0.05% carcinogen, or 0.05% sodium barbital, or both, for a period of two weeks, while controls received only the basal diet. Animals with hyperplastic nodules were obtained after 15 weeks of interrupted carcinogen feeding (1).

Assays

UDP glucuronyltransferase was measured using the decrease of absorbance at 410 nm on a Gilford 2000 spectrophotometer with *p*-nitrophenol as acceptor substrate according to a published procedure (5), with an important modification. We found that

ethanol, the suggested (5) solvent for *p*-nitrophenol in this assay, inhibits the transferase significantly. Generally ethanol inhibition becomes apparent at concentrations of 0.2 M and higher. Therefore, *p*-nitrophenol was added as an aqueous solution. The reaction was linear for 30 minutes at protein concentrations of 0.6-1.2 mg microsomal protein/reaction.

Microsomes were stored frozen at –80 C until assay, and were made micellar by sonification at 30 W for 30 seconds using a Wave-Line Sonifier at 0 C, just before assay. Uridine diphosphate glucuronic acid, *p*-nitrophenol, Tris, and malic acid were obtained from Sigma Chemical Company. The carcinogen was obtained from Aldrich Chemical Company. Styrene oxide [7-^{3}H, 1 nCi/nmole] was obtained from Amersham Chemical Company.

Epoxide hydrase was assayed according to the technique of Oesch, Jerina and Daly (6) with modification. Microsomes were solubilized by incubation for 15 min at 37 C in 0.2% sodium deoxycholate, a concentration we found to be optimum for expression of several types of liver microsomal epoxide hydrase. After extracting unreacted radioactive styrene oxide, the aqueous solution was allowed to remain at room temperature for 16 hours and then counted in Handifluor Scintillation Solution (Mallinckrodt Chemical Company). Microsomal protein was determined using the method of Lowry, *et al*. using bovine serum albumin as standard (7).

All results have been performed on three different rat livers, assayed in duplicate and the results expressed as the mean ± standard error.

RESULTS AND DISCUSSION

Microsomal UDP glucuronyltransferase and epoxide hydrase activities from liver of rats fed basal diet, diet plus 2-AAF, diet plus barbital, or diet including both drugs are listed in Table 1. Although both compounds induce both enzymes, 2-AAF is about twofold better an inducer of both activities than is barbital. Moreover, feeding diets with both barbital and 2-AAF appear to significantly reduce the level of glucuronyltransferase compared to the level obtained from livers of rats fed 2-AAF alone (compare 1.4-fold for the combination versus 2.3-fold for 2-AAF fed transferase — Table 1). This reduction is not apparent when one compares epoxide hydrase levels for 2-AAF feeding versus 2-AAF plus barbital feeding (Table 1). One explanation for this difference in response between enzymes which should be linked in detoxification metabolism, is the known multiple enzyme forms (isoenzymes) of UDP glucuronyltransferase (8, 9). Barbital may "override" any signal from 2-AAF, and induce a specific isoenzyme (giving 1.3-fold induction) while suppressing the more active *p*-nitrophenol transferase induced by 2-AAF. A recent report describes the physical separation of *p*-nitrophenol UDP glucuronyltransferase from estrone transferase (10).

Table 1 also lists the increase in both *p*-nitrophenol UDP glucuronyltransferase and styrene oxide hydrase in 2-AAF-induced hyperplastic nodules compared to normal

TABLE 1. *Effect of Dietary 2-AAF and Barbital on Hepatic* p-*Nitrophenol UDP Glucuronyltransferase and Styrene Oxide Hydrase Activities*

	p-Nitrophenol UDP Glucuronyltransferase (nmol/min/mg protein)	Epoxide Hydrase[a] (nmol/min/mg protein)
Basal	5.0 ± 0.8	3.3 ± 0.1
2-AAF	11.3 ± 1.0 (2.3)[b]	10.1 ± 0.4 (3.1)[b]
Barbital	6.4 ± 0.4 (1.3)	5.5 ± 0.3 (1.7)
2-AAF and barbital	7.1 ± 0.6 (1.4)	11.7 ± 0.4 (3.5)
2-AAF-induced hyperplastic nodule	21.8 ± 4.5 (4.4)	15.0 ± 0.6 (4.5)

[a]Epoxide hydrase was assayed at pH 7.4 in order to compare it to transferase, which has optimum at pH 7.4. The ratio of epoxide hydrase activities are similar at pH 7.4 or 9.0, its optimum pH.
[b]Fold increase relative to basal diet. Rats were fed diets containing 0.05% 2-AAF or barbital or both for 14 days.

liver. Both enzymes appear induced significantly more (4.4 and 4.5 fold) than in the liver microsomes from animals fed diet with carcinogen for two weeks (2.3 and 3.1 fold). Transferase is equal to or greater than hydrase in nodules as well as liver microsomes of animals fed 2-AAF for two weeks. This is consistent with a metabolic response by liver to enhance carcinogen detoxification by forming 2-AAF ring epoxides which are rapidly hydrolyzed to dihydrodiols and then conjugated to glucuronic acid prior to excretion (A, B, and C —Figure 1). This would tend to decrease the availability of the parent carcinogen, 2-AAF, for formation of the more toxic *N*-oxidized metabolites of 2-AAF (D, E and F — Figure 1). The high level of detoxifying enzymes in hyperplastic nodules is consistent with the prediction of Farber's (11) that preneoplastic tissues gain selective advantage through an alteration in their metabolism of carcinogen which protects them from metabolite toxicity relative to the more normal surrounding tissue. Whether or not the detoxification enzymes could be further regulated by diet or drugs to affect the outcome of 2-AAF hepatocarcinogenesis remains to be tested.

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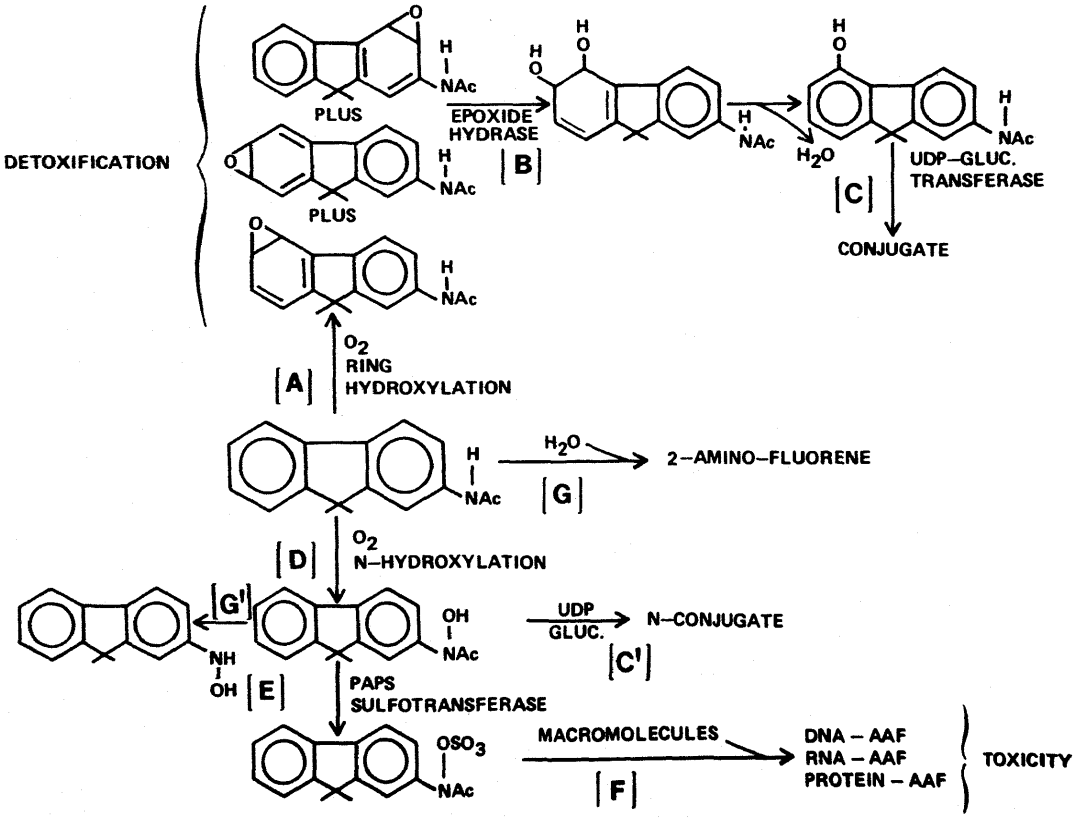

FIGURE 1. Proposed scheme for 2-AAF detoxification or metabolism to toxic compounds.

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