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AGE- AND SEX-RELATED DIFFERENCES IN ACTIVATION OF THE CARCINOGEN 7-HYDROXYMETHYL-12-METHYLBENZ[*a*]ANTHRACENE TO AN ELECTROPHILIC SULFURIC ACID ESTER METABOLITE IN RATS

POSSIBLE INVOLVEMENT OF HYDROXYSTEROID SULFOTRANSFERASE ACTIVITY

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Abstract—Metabolic activation of 7-hydroxymethyl-12-methylbenz[*a*]anthracene (HMBA) and related hydroxymethyl polycyclic aromatic hydrocarbons to electrophilic and mutagenic sulfuric acid esters has been demonstrated previously (Watabe *et al.*, In: *Xenobiotic Metabolism and Disposition* (Eds. Kato R, Estabrook RW and Cayen MN), pp. 393–400. Taylor & Francis, London, 1989). In the present study, the rat hepatic sulfotransferase activity catalyzing the formation of such reactive sulfuric acid esters was inhibited strongly by dehydroepiandrosterone, a typical substrate for hydroxysteroid sulfotransferases (HSSTs). Pentachlorophenol, a potent phenol sulfotransferase inhibitor, had little effect in this regard. A marked sex difference was observed for the hepatic cytosolic sulfotransferase activity for HMBA in rats. This sex difference was age-related; no significant difference was observed in preweanling rats, whereas in adult rats female rat liver showed a much higher enzyme activity. These age- and sex-related differences in the sulfonation of HMBA reflect the regulation of HMBA sulfotransferase activity by gonadal hormones as previously demonstrated with HSSTs. Thus, pretreatment with estradiol benzoate significantly enhanced the sulfotransferase activity for HMBA in both male and female rats, ($P < 0.01$ and $P < 0.05$ respectively), whereas testosterone propionate pretreatment decreased this activity. Castration of male rats increased the HMBA sulfotransferase activity 2- to 3-fold compared with that in control animals. By contrast, ovariectomy reduced the enzyme activity 38% in females. These results imply that rat liver HSST activity is responsible for the sulfonation of HMBA. Intraperitoneal injection of HMBA (0.25 μmol/g body wt) into infant rats produced benzylic DNA adducts in the liver which were chromatographically identical with those obtained from incubations of HMBA with deoxyguanosine and deoxyadenosine in the presence of hepatic cytosolic sulfotransferase activity. Intraperitoneal administration of sodium 7-sulfooxymethyl-12-methylbenz[*a*]anthracene resulted in much higher levels of these adducts and the deoxycytidine adduct in the liver DNA than did an equimolar amount of the parent hydroxymethyl hydrocarbon. The levels of hepatic benzylic DNA adducts formed from HMBA were reduced markedly by pretreatment of rats with dehydroepiandrosterone, a strong inhibitor of hepatic sulfotransferase activity for this hydrocarbon.

Sulfonation, like other metabolic conjugation reactions, has been generally regarded as a detoxication process for a variety of xenobiotics. In recent years, however, there has been an accumulation of experimental evidence that, in some cases, sulfo-conjugation plays an important role in metabolic toxication [1]. Thus, certain drugs and chemical carcinogens become chemically reactive upon sulfonation, thereby exerting toxic and carcinogenic effects. This was first noted in the hepatic sulfonation of *N*-hydroxy-2-acetylaminofluorene (N-OH-AAF‡) in the rat [2, 3]. Similar findings were later noted for *N*-hydroxyphenacetin and *N*-nitrosoethanolamine in the rat [4, 5]. Data from recent studies in our laboratory on several carcinogenic alkenylbenzenes, 4-aminoazobenzene, 2-acetylaminofluorene and their proximate hydroxy metabolites have provided strong evidence that sulfuric acid esters of these compounds are principal ultimate electrophilic and carcinogenic metabolites in the livers of infant male B6C3F$_1$ mice [6–9]. The chemical reactivity of these sulfuric acid ester metabolites resides in their ability to release sulfate ion as a leaving group with consequent formation

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‡ Abbreviations: N-OH-AAF, *N*-hydroxy-2-acetylaminofluorene; PAHs, polycyclic aromatic hydrocarbons; PAPS, 3′-phosphoadenosine-5′-phosphosulfate; HMBA, 7-hydroxymethyl-12-methylbenz[*a*]anthracene; SMBA, 7-sulfooxymethyl-12-methylbenz[*a*]anthracene; HMBP, 6-hydroxymethylbenzo[*a*]pyrene; PCP, pentachlorophenol; PSTs, phenol sulfotransferases; DHEA, dehydroepiandrosterone; HSSTs, hydroxysteroid sulfotransferases; Guo, guanosine; dGuo, deoxyguanosine; dAdo, deoxyadenosine; dCyd, deoxycytidine; TP, testosterone propionate; EB, estradiol benzoate; MOPS, 3-(*N*-morpholino)propane sulfonate; and DMSO, dimethyl sulfoxide.

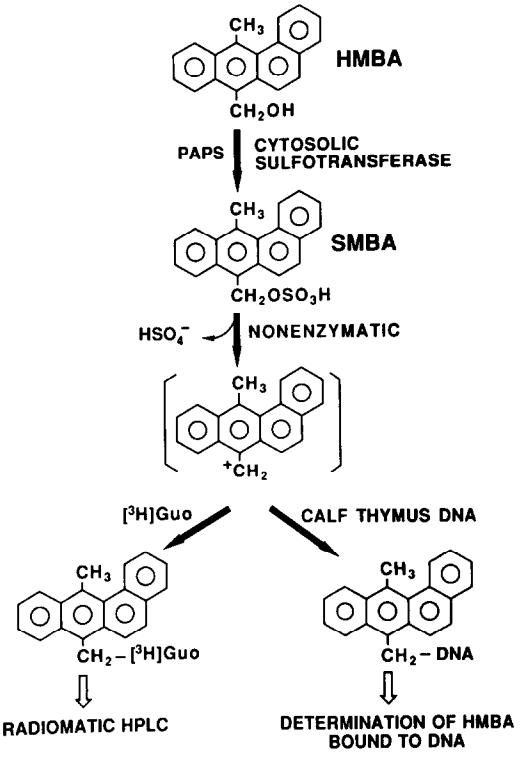

Fig. 1. Schematic representation of sulfotransferase activity assays for HMBA.

of resonance-stabilized benzylic carbocations or arylnitrenium ions.

Although several isozymes of cytosolic sulfotransferases have been found to be capable of esterifying various substrates including phenols, hydroxysteroids, carbohydrates and other important physiological biomolecules, the characteristics of the enzymes responsible for the formation of electrophilic sulfuric acid esters have not been well studied. An enzyme which catalyzes the formation of a carcinogenic sulfuric acid ester metabolite of N-OH-AAF has been purified to homogeneity from rat liver cytosol [10]. Cytosolic sulfotransferase activities for 1′-hydroxysafrole [6] and N-OH-AAF [9] are inhibited effectively by pentachlorophenol (PCP), a potent inhibitor of phenol sulfotransferases (PSTs) [11]. However, the sulfotransferases that sulfonate the hydroxymethyl polycyclic aromatic hydrocarbons (PAHs) are not much affected by this phenol, but are rather strongly inhibited by dehydroepiandrosterone

Table 1. PAPS-dependent sulfonation of HMBA by rat liver cytosol

Sulfo-group donor	Sulfotransferase activity*
PAPS	352
ATP and K_2SO_4	100
None	0

* Sulfotransferase activity for HMBA in the adult female rat liver cytosol was determined as described under Materials and Methods using [^{3}H]Guo as a nucleophilic acceptor. The enzyme activity is expressed as pmol of HMBA-[^{3}H]Guo adduct/mg cytosolic protein/30 min.

Table 2. Effects of PCP and DHEA on hepatic sulfotransferase activity for HMBA in rats

Inhibitor	Concn (μM)	Sulfotransferase activity	% Inhibition
None		342 ± 41	
PCP	100	292 ± 77	15
DHEA	100	113 ± 23	67

Liver cytosols from 12-day-old male rats were assayed for sulfotransferase activity for HMBA in the presence and absence of sulfotransferase inhibitors. Incubation conditions and other experimental details are as described in the text. Enzyme activity is expressed as pmol of the guanosine adduct produced/mg cytosolic protein/20 min. Values are the means ± SD of determinations on three animals.

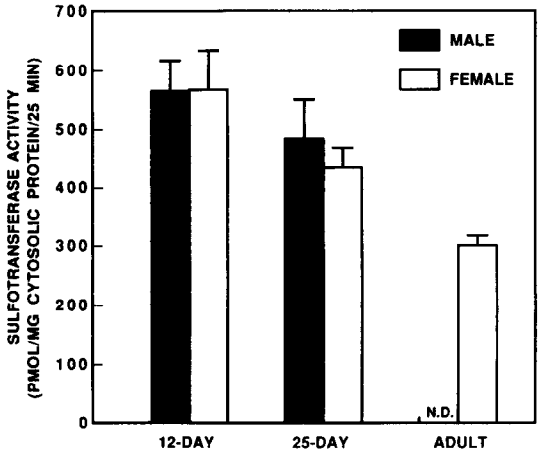

Fig. 2. Age-related sex differences in rat hepatic cytosolic sulfotransferase activity for HMBA. The enzyme assays were performed as described in Materials and Methods. The enzyme activities are given in pmol of HMBA-[^{3}H]Guo adduct formed/mg cytosolic protein/25 min. Bars represent the means of determinations on three separate pools of two preweanling rat livers or on three individual livers from adult rats (3- to 4-month-old). The vertical lines on the bars indicate one SD. N.D., not detectable.

(DHEA) [12–15], a typical substrate for hydroxysteroid sulfotransferases (HSSTs) [16]. A remarkable feature of rat liver HSSTs is the significant sex differences in their activities; adult female rats have a much higher enzyme activity than males [17–19]. A similar pattern of sex differences was observed in our recent studies on the 3′-phosphoadenosine-5′-phosphosulfate (PAPS)-dependent sulfonation of the carcinogen 6-hydroxymethylbenzo[*a*]pyrene (HMBP) by rat liver cytosol [14], indicating that the sulfotransferase activity for this hydrocarbon and possibly for other carcinogenic hydroxymethyl PAHs may be under the hormonal control analogous to that noted for HSSTs. Preliminary studies on 7-hydroxymethyl-12-methylbenz[*a*]anthracene (HMBA) also demonstrated the sex-related difference (female ≫ male) in the formation of an electrophilic sulfuric acid ester metabolite from this hydrocarbon by liver cytosols from adult rats [13]. To clarify the endocrine control of enzymatic sulfonation of hydroxymethyl

Table 3. Effect of administration of gonadal hormones on hepatic sulfotransferase activities in male and female rats

Treatment	Body wt (g)	Liver wt (g)	Sulfotransferase activity for HMBA	Sulfotransferase activity for *p*-Nitrophenol
Male				
TO	117 ± 6	4.9 ± 0.1	185 ± 77	19.6 ± 2.2
TP	133 ± 15	5.4 ± 0.3	44 ± 7*	21.4 ± 2.9
EB	106 ± 8	5.2 ± 0.2	499 ± 78†	9.4 ± 0.6†
Female				
TO	117 ± 7	4.6 ± 0.2	239 ± 55	14.4 ± 0.9
TP	121 ± 6	5.1 ± 0.3	92 ± 25†	19.4 ± 2.0†
EB	96 ± 5	5.1 ± 0.2	406 ± 75*	10.2 ± 0.4†

Male and female Sprague–Dawley rats were treated with testosterone propionate (TP) or estradiol benzoate (EB) as described in Materials and Methods. Control animals received the trioctanoin (TO) vehicle only. Enzyme activity for HMBA is expressed as pmol guanosine adduct formed/mg cytosolic protein/25 min and phenol sulfotransferase activity as nmol *p*-nitrophenol sulfate formed/mg cytosolic protein/10 min. Values are means ± SD (N = 4).

* Significantly different from the corresponding control ($P < 0.05$).

† Significantly different from the corresponding control ($P < 0.01$).

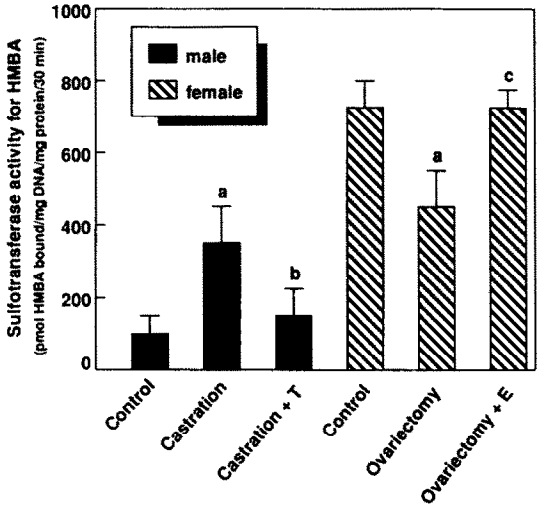

Fig. 3. Effect of gonadectomy and supplement of sex steroids on the hepatic sulfotransferase activity for HMBA in rats. Experomental details for the treatment of animals are described in Materials and Methods. The enzyme activity was determined by using calf thymus DNA as a nucleophilic acceptor and is given as pmol of HMBA residues covalently bound/mg DNA/mg cytosolic protein/30 min. Key: (a) significantly different from the respective controls, $P < 0.01$; (b and c) significantly different from the corresponding gonadectomy alone at the levels of $P < 0.05$ and $P < 0.005$ respectively. T, 5α-dihydrotestosterone; E, 17α-ethynylestradiol.

hydrocarbons, we have examined the effect of gonadectomy and/or sex hormone pretreatment on the hepatic cytosolic sulfotransferase activity for HMBA in rats. The role of sulfotransferase activity in the metabolic activation of HMBA in rat liver *in vivo* was also investigated in the present study.

MATERIALS AND METHODS

Chemicals. HMBA and [^{3}H]HMBA (>97% radiochemical purity) were prepared as described previously [20, 21]. 7-Sulfooxymethyl-12-methylbenz[*a*]anthracene (SMBA) was synthesized as a sodium salt in a manner similar to that adopted for the synthesis of sulfuric acid esters of other hydrocarbons [15]. PAPS was synthesized [22, 23] or obtained commercially from the Sigma Chemical Co. (St. Louis, MO). [5′-^{3}H]Guanosine (Guo) and [8-^{3}H]Guo were purchased from the Amersham Corp. (Arlington Heights, IL) and ICN Radiochemicals (Irvine, CA) respectively. PCP was a product of the Fluka Chemical Co. (Hauppaga, NY). Calf thymus DNA, 3-(*N*-morpholino)propane sulfonate (MOPS), DHEA and other steroids were supplied by the Sigma Chemical Co. Trioctanoin was obtained from Pfaltz & Bauer, Inc. (Stamford, CT). The other chemicals used were of reagent grade.

Instrumentation. Reverse-phase HPLC was accomplished with an Ultrasphere Octadecylsilane column (5 μm, 4.6 mm × 250 mm; Alltech Associates Inc., Deerfield, IL) on a model 660 solvent programmer and two model 6000A solvent delivery systems (Waters Associates, Milford, MA). A Waters model 440 absorbance detector (254 nm), a Flo-One/β model 1C radiomatic flow detector (Radiomatic Instrument & Chemical Co., Tampa, FL), and/or a Schoeffel FS 970 LC fluorescence detector were connected as required.

Animal treatment. Male and female Sprague–Dawley rats were obtained from Harlan Sprague–Dawley (Madison, WI) and bred in our laboratory. All of the breeding and experimental animals were maintained on Wayne Breeder Blox pellets (Allied Mills, Inc., Chicago, IL) and water *ad lib.* For hormone treatment, groups of 28-day-old rats were injected subcutaneously each morning with 1.0 mg of testosterone propionate (TP) or 0.2 mg of estradiol benzoate (EB) in 0.2 mL of trioctanoin, alternating between right and left hind legs for 12 days. Control animals received the vehicle only under the same conditions. Castration and ovariectomy were performed at birth. After 2 weeks of recovery, animals were given a daily subcutaneous injection of 5α-dihydrotestosterone (1.5 mg/kg body wt) or 17α-ethynylestradiol (60 μg/kg) in 0.1 mL propylene

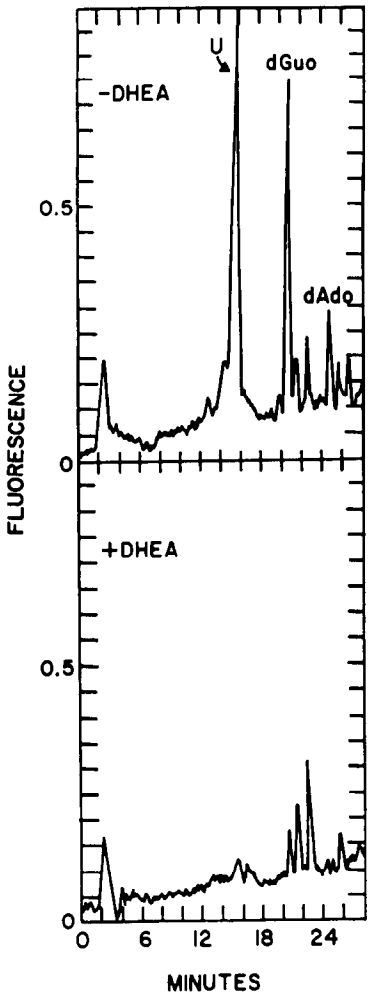

Fig. 4. Effect of DHEA pretreatment on the benzylic DNA adduct formation from HMBA in rat liver. Male Sprague–Dawley rats (12-day-old) were injected i.p. with DHEA (0.5 μmol/10 μL trioctanoin/g body wt) or vehicle only 1 hr before a dose of HMBA (0.25 μmol/g body wt). Hydrolysates of ~1 mg DNA were analyzed by HPLC with a 25-min linear gradient of 60–100% methanol and adducts were detected by their fluorescence (excitation, 361 nm; emission, 418 nm). The fluorescence was measured on an arbitrary linear scale. dGuo and dAdo represent deoxyguanosine and deoxyadenosine adducts respectively. These adducts were characterized previously by Watabe *et al.* [32]. U denotes an uncharacterized adduct.

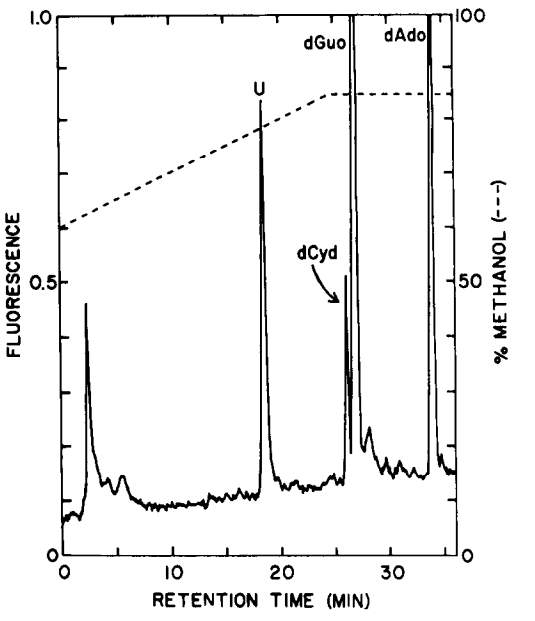

Fig. 5. HPLC profile of enzymatic hydrolysate of hepatic DNA from an infant male rat injected i.p. with SMBA (0.1 μmol/5 μL DMSO/g body wt). Hepatic benzylic DNA adducts were analyzed as described in the legend to Fig. 4 except that the solvent system was a linear gradient from 60 to 85% methanol in water (25 min) followed by isocratic elution at 85% methanol (15 min). The amount of DNA hydrolysate chromatographically analyzed was ~0.5 mg in terms of DNA equivalents. dCyd represents the deoxycytidine adduct; abbreviations for other adducts are the same as in Fig. 4.

glycol or only the vehicle for 4 weeks. At the end of the study period, the livers were rapidly removed, weighed, homogenized in 3 vol. of ice-cold 0.154 M KCl–50 mM Tris–HCl buffer (pH 7.4), and centrifuged as described previously [14]. The final cytosolic fractions were assayed for sulfotransferase activity. The cytosolic proteins were determined according to the method of Lowry *et al.* [24].

Enzyme assays. Sulfotransferase activity for HMBA was assayed using either [^{3}H]Guo or calf thymus DNA as a nucleophilic acceptor (see Fig. 1). Unless otherwise indicated, the standard incubation mixture in a final volume of 125 μL contained HMBA (0.25 μmol) in 5 μL DMSO, [^{3}H]Guo (0.5 μmol), PAPS (0.25 μmol), liver cytosol (equivalent to 0.5 to 0.75 mg protein), $MgCl_2$ (2 μmol), and MOPS buffer, pH 7.4 (20 μmol). In some experiments, PAPS was replaced by ATP (2 μmol) and K_2SO_4 (2 μmol). For inhibition studies, PCP and DHEA in 5 μL DMSO were added to the incubation mixtures. Incubation was performed at 37° for 20–30 min and terminated by rapid cooling on ice. The incubation mixtures were extracted twice with 150 μL benzene to remove unreacted HMBA. The aqueous phase was further extracted twice with 175–200 μL of $CHCl_3$:*n*-propanol (7:3, v/v). The combined organic layer was washed with 250 μL of water and evaporated to dryness. The residue was dissolved in methanol–water for HPLC analysis with a 25-min linear gradient of 60% methanol in water to 100% methanol at a flow rate of 1 mL/min. The HMBA–[^{3}H]Guo adduct was monitored by both UV absorbance and radioactivity. Alternatively, calf thymus DNA was included as a nucleophilic acceptor to capture the labile reactive sulfuric acid ester of [^{3}H]HMBA (sp. act. 0.01 to 0.04 Ci/mmol) in incubations with liver cytosol and PAPS, and the resultant covalent binding was determined as previously reported [14]. Assays for phenol sulfotransferase activity were accomplished by the published method [25]. Statistical significance was determined by Student's unpaired *t*-test; $P < 0.05$ was considered significant.

Effect of DHEA pretreatment on hepatic benzylic DNA adduct formation from HMBA in rats. Infant male rats (12-day-old) were injected i.p. with DHEA

(0.5 μmol/10 μL trioctanoin/g body wt) or the same volume of the vehicle 1 hr prior to the administration (i.p.) of 0.25 μmol HMBA/5 μL DMSO/g body wt. Animals were killed 16 hr later, and their hepatic DNA was isolated by the method of Irving and Veazey [26]. DNA quantitation, enzymatic digestion, and extraction of benzylic DNA adducts were carried out as reported previously [14]. Extracts of DNA hydrolysates were analyzed by HPLC under the same conditions as used in the above sulfotransferase assay for HMBA and the adducts were detected by their fluorescence at 361 nm (emission, 418 nm). The identity of the benzylic DNA adducts was confirmed by co-chromatography with the standard adducts prepared by reaction of SMBA with individual deoxyribonucleosides.

Formation of hepatic benzylic DNA adducts in rats treated with SMBA. Male rats (12-day-old) were injected either intraperitoneally or intravenously with 0.1 μmol SMBA/5 μL DMSO/g body wt. Animals were killed 6 hr after SMBA injection. Hepatic DNA was isolated, digested, and analyzed by HPLC as described above. For better separation of the benzylic DNA adducts, HPLC was performed with a linear gradient of 60 to 85% methanol for 25 min followed by 15-min isocratic elution at 85% methanol.

RESULTS

Sulfotransferase activity for HMBA in rat liver cytosol. The metabolic formation of an electrophilic sulfuric acid ester of HMBA was examined by using a sulfotransferase assay system where the short-lived sulfuric acid ester metabolite was trapped by a nucleophilic acceptor such as [^{3}H]Guo or calf thymus DNA (Fig. 1). Both cytosol and the sulfo-group donor PAPS were required for the formation of this reactive ester; inactivation of cytosolic protein by heating or omission of PAPS from the incubation mixture resulted in no appreciable sulfotransferase activity for HMBA. When PAPS was replaced by ATP and K_2SO_4 to generate it, sulfotransferase activity for HMBA was also observed, but the enzyme activity was lower than that obtained by the direct addition of PAPS (Table 1). PCP (100 μM) only slightly inhibited the sulfotransferase activity for HMBA, whereas DHEA, a typical substrate for hydroxysteroid sulfotransferases, strongly inhibited the activity at the same concentration (Table 2).

Age-related sex differences were observed for the cytosolic sulfotransferase activity for HMBA (Fig. 2). The activity was not significantly different between male and female rats before weaning, but declined with aging much faster in males. As a result, no appreciable activity was observed in the hepatic cytosols from adult male rats (Fig. 2). These striking sex differences in sulfotransferase activity for HMBA in adult rats appear to be associated with regulation of the enzyme activity by gonadal hormones as previously noted by others for steroid sulfotransferases [18, 19, 27] and bile salt sulfotransferases [28, 29]. Pretreatment of rats with the female sex hormone EB markedly enhanced the enzyme activity responsible for the sulfonation of HMBA, whereas TP pretreatment lowered it (Table 3). On the contrary, PST activities in the hepatic cytosols of the same animals were modified in the opposite manner by the hormone treatments (Table 3) consistent with previous results reported by other investigators [30, 31]. To better understand the role of gonadal hormones in regulating the sulfotransferase activity for HMBA, effects of gonadectomy on the sulfonation of HMBA were examined. As shown in Fig. 3, feminization of male rats by neonatal castration resulted in a significant increase in the HMBA sulfotransferase activity. Subsequent treatment of castrated male rats with testosterone decreased the above enzyme activity 53% compared with castration only (341 ± 111 vs 161 ± 79 pmol/mg DNA/mg protein/30 min, $P < 0.05$). In female rats, ovariectomy caused an effective reduction in the enzyme activity to a level 38% compared with controls (728 ± 79 vs 451 ± 116, $P < 0.01$). Replenishment of estradiol in ovariectomized female rats restored the cytosolic activity of HMBA sulfonation to a level similar to that of control animals (Fig. 3).

Formation of hepatic benzylic DNA adducts from HMBA and SMBA in rats. Since hepatic sulfotransferase activity for HMBA was inhibited by DHEA, it was interesting to determine its effect *in vivo* on the hepatic benzylic DNA adduct formation from HMBA. As represented in Fig. 4, hydrolysates of hepatic DNA from rats without DHEA pretreatment contained three adducts, two of which, designated as dGuo and dAdo, were chromatographically identical with the authentic adducts obtained by reaction of SMBA with deoxyguanosine and deoxyadenosine respectively. These DNA adducts were characterized previously as benzylic adducts in which the 7-methylene carbon is covalently bound to the amino groups of the purine bases ([32], recently reviewed in Ref. 33). The identity of the third adduct (U) which eluted before the above benzylic adducts is under investigation. DHEA pretreatment significantly reduced the levels of all three adducts (Fig. 4, bottom).

Our previous data demonstrated that much larger amounts of the above DNA adducts were formed in the livers of rats and mice after intraperitoneal injection of the electrophilic sulfuric acid ester, SMBA [12]. In the present study, an additional adduct was found in hepatic DNA from SMBA-treated rats (Fig. 5). This new adduct co-chromatographed with a standard prepared from reaction of SMBA with deoxycytidine. It is of interest that the labile ester SMBA ($T_{1/2}$, <1 min in aqueous media; Ref. 33), when administered i.p. to rats or mice, produced higher levels of hepatic DNA adducts than did the same dose of the parent hydroxymethyl hydrocarbon HMBA. Injection of SMBA intravenously into infant rats produced amounts of benzylic DNA adducts in the liver DNA similar to those obtained by i.p. injection (data not shown).

DISCUSSION

Hydroxylation of *meso*-methyl groups with subsequent formation of reactive benzylic esters bearing

a good leaving group such as sulfate, phosphate and acetate has been proposed by Flesher and Sydnor as a possible metabolic pathway in the activation of some carcinogenic methyl-substituted PAHs such as 7,12-dimethylbenz[*a*]anthracene and 6-methylbenzo[*a*]pyrene [34, 35]. Results from carcinogenicity and mutagenicity tests as well as metabolic studies *in vitro* have provided some support of this concept [34–38]. More definitive supporting data on the metabolic formation of such reactive esters *in vitro* have been published recently by Watabe and his colleagues [32, 33]. Their studies demonstrated the formation of reactive and mutagenic sulfuric acid ester metabolites from HMBA and a series of other carcinogenic hydroxymethyl hydrocarbons by rat liver cytosolic sulfotransferase activity. We have been interested in elucidating the sulfonation *in vivo* of several hydroxymethyl PAHs and the role of metabolically formed sulfuric acid esters in the tumorigenesis by these hydrocarbons. Our recent studies on HMBA, HMBP and other related hydrocarbons have shown that their electrophilic sulfuric acid ester metabolites play a major role in the formation of benzylic DNA adducts from the parent hydroxymethyl hydrocarbons in infant rats ([12–15], briefly reviewed in Ref. 39).

Although several sulfotransferase activities have been reported, consideration of the specificity of purified sulfotransferases with respect to their catalytic activity for several types of carcinogenic hydrocarbons has been limited. A sulfotransferase responsible for the formation of an electrophilic sulfuric acid ester of N-OH-AAF was purified to homogeneity from the rat liver [10], and its physical and catalytic properties were later attributed to aryl sulfotransferase IV [40], a group of well defined PSTs. It seems unlikely that hepatic PSTs are responsible for the conjugation of hydroxymethyl PAHs with sulfate since PCP, a well known inhibitor of PST activity [11], was not effective in this regard. On the contrary, DHEA which is a typical substrate for HSSTs strongly inhibited the sulfotransferase activity for HMBA (Table 2) and other related hydroxymethyl PAHs [13–15]. In consideration of the structural similarity between steroids and PAHs, hydroxy steroid sulfotransferases may catalyze the formation of electrophilic sulfuric acid esters from the aforementioned benzylic hydroxymethyl aromatic hydrocarbons. This assumption appears to be quite likely since Lyon and Jakoby [41] previously demonstrated the identity of HSSTs with alcohol sulfotransferases which were found to utilize a variety of alcohols as sulfate acceptors. Aryl sulfotransferase IV has also been reported to be capable of catalyzing the sulfonation of some benzylic alcohols in addition to phenols [42, 43], but its catalytic activity was found to be limited only to molecules of relatively small size such as 1-naphthalene methanol. Therefore, it is improbable that this enzyme catalyzes the sulfonation of hydroxymethyl PAHs which have relatively larger molecular sizes.

One of the most important characteristics which distinguish between HSSTs and PSTs is their opposite pattern of sex differences [30, 44, 45]; for the former enzymes, higher activity is observed in female rats whereas male rats show higher activity of the latter enzymes than females. Sulfotransferase activity for HMBA was predominant in adult female rats (Fig. 2) as in the case of HSSTs. The sex difference in sulfonation of HMBA appears to be associated with regulation of the sulfotransferase activity for this hydrocarbon by gonadal hormones, probably due to the stimulatory effects of estrogens and suppressing effects of androgens as previously suggested for hydroxysteroid sulfotransferases [27, 30]. Evidence for this assumption was further provided by comparing the cytosolic sulfotransferase activities in the livers of gonadectomized rats and control animals. Thus, castration elevated the HMBA sulfotransferase activity in male rats and ovariectomy decreased the enzyme activity in females (Fig. 3). Again, these results are in agreement with those previously demonstrated with HSSTs. The higher sulfotransferase activity for HMBA in female rats raises the possibility that they may be more susceptible to carcinogenesis by this hydrocarbon and 7,12-dimethylbenz[*a*]anthracene than males. The inhibition of sulfotransferase activity for HMBA by DHEA and the above characteristic sex differences similar to those of HSSTs provide evidence for the involvement of the HSST activity in the formation of an electrophilic sulfuric acid ester metabolite of this carcinogen. Similar findings were reported previously by Okuda and his co-workers with 5-hydroxymethylchrysene [46]. Interestingly, the sex-related differences in the hepatic cytosolic sulfotransferase activity for this hydrocarbon appear to be species dependent; 4- to 5-fold sex differences were observed in rats and mice while small differences were noted in guinea pigs and hamsters. Ogura *et al.* [47] have cloned a cDNA encoding a rat liver HSST which showed high catalytic activity for DHEA. Northern-blot analysis showed a marked sex difference (female $\gg$ male) in the expressed level of the mRNA for the predicted HSST protein. Immuno-blot analysis with the anti-serum raised against the purified HSST also indicated that liver cytosols from female rats contained a much higher level of the HSST protein than those from males. These results, at the molecular level, clearly explain the sex differences previously observed for the sulfonation of certain steroids in rats. It will be of interest to determine whether this enzyme is also catalytically active in sulfonating the hydroxymethyl PAHs as mentioned above. Multiple forms of HSSTs are known to exist [16, 41], and further study will be needed to fully identify the above purified sulfotransferase. Rat hepatic bile salt sulfotransferase I activity has been shown to be regulated by gonadal hormones in the same manner as the HSST activity [28, 29]. Recent studies by Barnes *et al.* [48] suggested the identity of this enzyme with the HSST 2 previously classified by Lyon *et al.* [16]. This conclusion was made on the basis of their amino acid sequence homology and the similar subunit molecular weight (~30 kD) as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. In addition, the HSST 2 activity with DHEA as a substrate was found to be inhibited competitively by an inhibitor of bile salt sulfotransferases [48]. A form of sulfotransferase capable of sulfonating

DHEA and other related steroids has also been recently purified 621-fold from human liver cytosols [49].

Injection of HMBA to infant rats produced the same benzylic adducts in the liver DNA as those formed by incubation of this hydrocarbon with calf thymus DNA in the presence of liver cytosolic sulfotransferase activity. The markedly reduced levels of these benzylic adducts in the hepatic DNA of rats pretreated with the sulfotransferase inhibitor DHEA suggest that the same enzyme activity may also be involved in the activation of HMBA to an electrophilic sulfuric acid ester metabolite *in vivo*. However, the possibility cannot be excluded that effects of DHEA on other physiological processes may also have affected the formation of the hepatic benzylic DNA adducts.

Despite the short half-life of SMBA in an aqueous solution (<1 min) [33], this electrophilic sulfuric acid ester was found to form much larger amounts of hepatic benzylic DNA adducts in rats and mice than the parent hydroxymethyl hydrocarbon when injected into these animals intraperitoneally [12]. This may be due to transport of this labile ester to the liver in a manner that permitted a sufficient fraction of the dose to survive the aqueous environment in the abdominal cavity and in the vascular transport to the liver and uptake by the liver cells. Since the route of administration may profoundly affect the pharmacokinetics of xenobiotics administered to animals, experiments were undertaken to compare the i.p. injection of SMBA with another route of administration. For this purpose, intravenous injection was included since it could avoid the problem of direct absorption of the short-lived sulfuric acid ester from the liver surface which might occur by intraperitoneal injection. However, the data indicate no significant difference in the pattern and amounts of hepatic benzylic DNA adducts produced from SMBA by these two routes of administration. These results suggest that the short-lived sulfuric acid ester can be systemically transported to the liver. Some carrier proteins in the systemic circulation such as serum albumin or plasma lipoprotein may sequester the electrophilic sulfuric acid ester through non-covalent interactions that stabilize this otherwise water-labile ester by protecting it from rapid hydrolysis, thereby transferring it to the liver where it produces the DNA adducts. An analogous assumption has been suggested recently [50–52] for the bay-region dihydrodiol epoxide of benzo[*a*]pyrene which is generally considered as an ultimate electrophilic and carcinogenic metabolite of this hydrocarbon. The life-span of this water-labile epoxide is increased in the presence of serum albumin [53] or microsomal lipid [54]. Since Kupffer cells in the liver are known to remove particulate materials including colloidal particles which originate from the blood, they may be able to take up presumed micellar complexes of the sulfuric acid ester of HMBA from the blood. Further studies will be necessary to test this possibility.

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