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DIFFERENTIAL RESPONSES OF MOUSE UDP-GLUCURONOSYLTRANSFERASES AND BETA-GLUCURONIDASE TO DISULFIRAM AND RELATED COMPOUNDS

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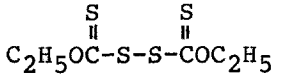
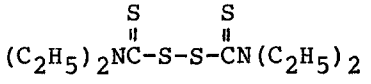
SUMMARY Studies on the induction of non-oxygenative detoxication enzymes in mice by anticarcinogenic thionosulfur compounds have been extended to include hepatic and pulmonary UDP-glucuronosyltransferases. Dietary administration of disulfiram and of bisethylxanthogen to female CD-1 mice enhanced microsomal glucuronidation of 4-methylumbelliferone, a characteristic GT_1 substrate, and of 4-hydroxybiphenyl, a GT_2 substrate. Latency of the activity toward 4-methylumbelliferone was not affected appreciably. Disulfiram also enhanced glucuronidation of 4-nitrophenol. Diethyldithiocarbamate was ineffective under the conditions used. These thionosulfur compounds caused no significant change in beta-glucuronidase activity measured in homogenates of 7 organs.

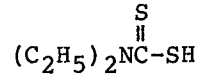
Bisethylxanthogen, disulfiram, and diethyldithiocarbamate are thionosulfur compounds that have been shown to protect rodents against chemical carcinogenesis and toxicity and to inhibit enzymes involved in the oxidative activation of some carcinogens [1-4]. Our studies, which are directed toward elucidating the effects of anticarcinogenic compounds on non-oxygenative detoxication enzymes, have shown that these 3 thionosulfur compounds induce high levels of glutathione transferase and DT-diaphorase activities in several mouse organs [5,6].



Bisethylxanthogen



Disulfiram



Diethyldithiocarbamate

Glucuronidation is another major pathway for non-oxygenative metabolism of many drugs, steroids, and toxic and carcinogenic chemicals [7,8]. It has

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been noted that rat hepatic glucuronidation of 4-nitrophenol is enhanced by disulfiram and diethyldithiocarbamate [9]. Rodent liver microsomes contain multiple UDP-glucuronosyltransferase [EC 2.4.1.17] isoenzymes that differ in inducibility and ontogeny, and in their activities toward specific substrates [10-12]. In the present investigation we have explored the effects of the dietary administration of bisethylxanthogen, disulfiram, and diethyldithiocarbamate on the activities of mouse liver microsomes in the glucuronidation of several substrates. These included 4-methylumbelliferone as a substrate of the Group 1 or late fetal cluster of UDP-glucuronosyltransferase activities, 4-hydroxybiphenyl as a representative substrate for the Group 2 or neonatal cluster of activities, and 4-nitrophenol which appears to be a less selective substrate [10-12]. The effects of the thionosulfur compounds on pulmonary microsomal UDP-glucuronosyltransferase activity were also examined. To determine whether the 3 anticarcinogenic compounds might alter the potential for the hydrolysis of glucuronides, we examined their effects on beta-glucuronidase [3.2.1.31] activity in mouse liver and 6 extrahepatic organs.

MATERIALS AND METHODS

Chemicals. Disulfiram (Aldrich Chemical Company, Milwaukee, WI) and bisethylxanthogen (a gift from Fike Chemicals, Nitro, WV) were recrystallized 5 times from cold ethanol before use. Sodium diethyldithiocarbamate from Eastman Organic Chemicals, Rochester, NY, was used without further purification. Sucrose (ultra pure grade) was from Schwarz/Mann, Spring Valley, NY. The following chemicals were from Sigma Chemical Company, St. Louis, MO: UDP-glucuronic acid triammonium salt, 4-methylumbelliferone, 4-methylumbelliferyl-beta-D-glucuronide, 4-hydroxybiphenyl, 4-nitrophenol, polyoxyethylene 20 cetyl ether (Brij 58), Lubrol PX, phenolphthalein glucuronic acid (as a neutralized 10 mM solution), and phenolphthalein.

Treatment of mice. Female CD-1 mice, 5 weeks old, were purchased from Charles River Breeding Laboratories, Portage, MI and Wilmington, MA. They were housed in stainless steel wire cages and received ad libitum a basal diet of Purina Laboratory Chow (Ralston Purina, St. Louis, MO) in powdered form. After 3 to 5 weeks of acclimatization, the mice were randomized by weight and given experimental diets containing bisethylxanthogen, disulfiram, or diethyldithiocarbamate. These compounds were added at 20 μmoles per gram of diet, which is within the concentration range used by Wattenberg et al. [2,3]. Control groups continued to receive the basal diet. After 14 days on these diets, the mice were killed by cervical dislocation and organs were excised immediately and processed as described previously [13].

Preparation of tissue homogenates and microsomes. All steps were carried out at 0-4°C. Tissue homogenates for determination of beta-glucuronidase activities were prepared in a Duall homogenizer, using 20-100 mg of minced tissue in distilled water (1 ml of water per 20 mg of tissue). Duplicate 10 μl portions of each homogenate were assayed for protein concentration after incubation with 1 N NaOH at 37°C for 20 min by the method of Lowry et al. [14]. For the preparation of microsomes, livers and lungs were homogenized in 0.25 M sucrose (3 ml per g of tissue). Liver and lung homogenates were centrifuged at 27,000 X g and at 9,800 X g, respectively. Microsomes were prepared as described previously [15], washed twice by suspension in 0.25 M sucrose, suspended in a volume of 0.25 M sucrose based upon the weight of the tissue from which they were derived (0.5 ml per g of liver or 1 ml per gm of lung), aliquotted, frozen in liquid nitrogen, and stored at -70°C. The

protein concentrations of the microsomal suspensions were determined by the method of Lowry et al. [14].

Measurement of UDP-glucuronosyltransferase and beta-glucuronidase activities. 4-Methylumbelliferone, 4-hydroxybiphenyl, and 4-nitrophenol were used as substrates. A 20 mM solution of UDP-glucuronic acid triammonium salt, adjusted to pH 7.4 to 7.5 with NaOH, was used in all assays. The glucuronidation of 4-nitrophenol by liver microsomes was measured as described by Burchell and Weatherill [16]. Lubrol PX was included at a final concentration of 0.04% in the 0.25 ml incubation mixture. Residual 4-nitrophenol was measured spectrophotometrically at 405 nm using a molar extinction coefficient of 18,100 [16]. Reactions were linear for at least 30 min using 0.15 to 0.75 mg of microsomal protein. Measurements of the glucuronidation of 4-methylumbelliferone and 4-hydroxybiphenyl were based on methods described by Bock et al. [12]. The 0.5 ml reaction mixtures contained 100 mM Tris chloride (pH 7.4), 5 mM $MgCl_2$, 0.5 mM 4-methylumbelliferone (added in 25 µl of dimethylsulfoxide) or 0.5 mM 4-hydroxybiphenyl (added in 25 µl of methanol), 50 to 70 µg of microsomal protein, and 3 mM UDP-glucuronic acid. Brij 58 (0.16 mg/ml) was included in the assay systems as specified below. The mixtures were equilibrated to 37°C for 5 min before addition of the UDP-glucuronic acid to start the reaction. After 5 to 10 min (liver microsomes) or 15 min (lung microsomes) the reactions were stopped by addition of 0.5 ml of 0.5 M $HClO_4$. After centrifugation for 20 min at 1,900 X g and extraction of the supernatants with 1-2 ml of chloroform [12], 0.5 ml of the aqueous phase was diluted with 1.5 ml of 1.6 M glycine (pH 10.3) and fluorescence was measured in a Farrand MK-1 spectrofluorometer. With 4-methylumbelliferone as the substrate, measurements were at 365 nm with excitation at 315 nm. Calibration was with 4-methylumbelliferyl-beta-D-glucuronide. With 4-hydroxybiphenyl as the substrate, fluorescence was measured at 325 nm with excitation at 290 nm and calibration utilized Folin-Ciocalteau reagent [14] to measure residual substrate [17]. Under the assay conditions described, product formation was proportional to the amount of microsomal protein used and to the time of incubation. UDP-glucuronosyltransferase activities were corrected for nonenzymatic rates measured in the absence of UDP-glucuronic acid. Measurement of beta-glucuronidase activities was based on the method of Talalay et al. [18]. Stoppered assay tubes containing 0.2 ml of homogenate, 0.16 ml of 0.2 M sodium acetate at pH 4.5, and 0.04 ml of phenolphthalein glucuronide were incubated at 37°C for 60 min. Each tube then received 1.2 ml of 0.4 M alkaline glycine solution (containing 1.63 g of glycine and 1.27 g of NaCl per 100 ml, and adjusted to pH 10.45 with NaOH). After centrifugation of the samples at 12,350 X g for 10 min, the concentration of free phenolphthalein in each supernatant was determined from its absorbance at 552 nm, using a standard curve developed with commercial phenolphthalein.

RESULTS

Disulfiram and bisethylxanthogen exhibited similar effectiveness in inducing liver microsomal UDP-glucuronosyltransferase activities toward 4-hydroxybiphenyl and 4-methylumbelliferone (Table I). Disulfiram also increased activity toward 4-nitrophenol, whereas diethyldithiocarbamate had no significant effect on any of the activities examined. The latency of microsomal UDP-glucuronosyltransferase activity toward 4-methylumbelliferone was not altered appreciably by pretreatment of the mice with bisethylxanthogen, disulfiram, or diethyldithiocarbamate. Measurements under conditions of maximal activation by Brij 58, and in the absence of detergent, showed that the latency of this activity in the liver microsomes essentially unaffected, being 74% for the untreated mice and ranging from 66% to 72% for mice treated with

TABLE I. EFFECTS OF DIETARY ADMINISTRATION OF THIONOSULFUR COMPOUNDS ON MOUSE HEPATIC UDP-GLUCURONOSYLTRANSFERASE ACTIVITIES

Substrate	UDP-glucuronosyltransferase activity (nmol/min per mg of microsomal protein, ± S.E.M.[a])			
	Control diet	Bisethyl-xanthogen	Disulfiram	Diethyldithio-carbamate
4-Methylumbelliferone				
With Brij 58[b]	38.7 ± 2.7	64.3 ± 4.9 (p<0.001)	52.7 ± 4.5 (p<0.05)	38.0 ± 3.9
Without detergent	10.0 ± 1.2	19.7 ± 1.1 (p<0.001)	14.9 ± 1.3 (p<0.02)	12.8 ± 1.7
4-Hydroxybiphenyl	111 ± 13	155 ± 17 (p<0.10)	150 ± 10 (p<0.05)	103 ± 11
4-Nitrophenol	7.1 ± 0.6	9.2 ± 1.0	10.6 ± 1.0 (p<0.05)	6.8 ± 0.7

[a] n = 6 to 10 with different substrates. Assays were run in duplicate.
[b] Brij 58 was used as an activator, as described under Materials and Methods.

the thionosulfur compounds. Attempts to measure bilirubin UDP-glucuronosyltransferase were unsuccessful because the binding of bilirubin to the microsomes precluded accurate measurements of the very low activity toward this substrate.

With 4-methylumbelliferone as the substrate, the specific activities of pulmonary microsomes from untreated mice were 38% and 19% relative to liver microsomes when assayed in the absence and presence of Brij 58, respectively. Other investigators have noted that mouse lung microsomes have little or no activity in the glucuronidation of 4-hydroxybiphenyl [12], and that the presence of pulmonary activity toward 4-nitrophenol is species-dependent [8]. We observed very low activity toward both of these substrates and did not examine them further. The effects of the thionosulfur compounds on pulmonary microsomal UDP-glucuronosyltransferase activity toward 4-methylumbelliferone are shown in Figure 1. The activity, measured in the presence of Brij 58, was not significantly increased by diethyldithiocarbamate, but almost doubled in response to bisethylxanthogen and disulfiram.

Beta-glucuronidase activity was measured in homogenates of seven organs from untreated mice and from mice treated with bisethylxanthogen, disulfiram, or diethyldithiocarbamate. Table II summarizes the specific activities of beta glucuronidase in organs from untreated mice and from mice that had received the dietary thionosulfur compounds.

DISCUSSION

The enhanced glucuronidation of both 4-methylumbelliferone and 4-hydroxybiphenyl by liver and lung microsomes from mice treated with either bisethylxanthogen or disulfiram suggests that these thionosulfur compounds induced

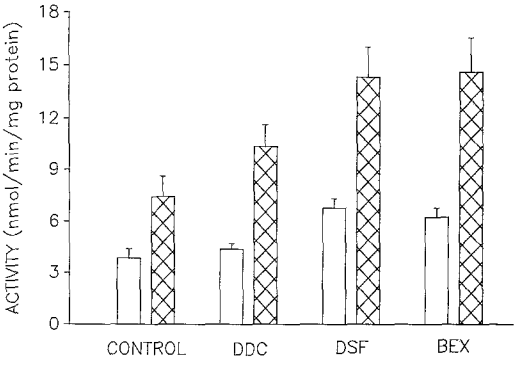

FIG. 1. EFFECTS OF DIETARY THIONOSULFUR COMPOUNDS ON 4-METHYLUMBELLIFERONE GLUCURONIDATION BY PULMONARY MICROSOMES. Mice received the control diet or the diethyldithiocarbamate (DDC), disulfiram (DSF), or bisethylxanthogen (BEX) diet for 14 days and pulmonary microsomes were assayed for UDP-glucuronosyltransferase activity toward 4-methylumbelliferone, as described in Materials and Methods. The mean specific activities are shown, ± S.E.M. (n = 6-7). Open bars: activities in the absence of detergent. Crosshatched bars: activities measured with activation by Brij 58. All specific activities shown for disulfiram and bisethylxanthogen groups were significantly elevated relative to the corresponding activities shown for the control group ($p<.01$).

both Group 1 (GT_1) and Group 2 (GT_2) UDP-glucuronosyltransferase activities. GT_1 activity is inducible in livers of aryl hydrocarbon responsive strains of mice by 3-methylcholanthrene [12,19] and is believed to be regulated, at least in part, via the "Ah" locus [19,20]. The degree of induction of hepatic GT_1 activity that we observed in bisethylxanthogen treated CD-1 mice was equal to that observed by Bock et al. [12] in livers of C57BL mice treated with 3-methylcholanthrene.

Marselos et al. [9] reported induction of liver microsomal glucuronidation of 4-nitrophenol in Wistar rats by disulfiram and by diethyldithiocarbamate [9]. In the present investigation this activity, which is catalyzed

TABLE II. BETA-GLUCURONIDASE ACTIVITIES OF ORGAN HOMOGENATES FROM MICE FED CONTROL DIET OR DIETS CONTAINING THIONOSULFUR COMPOUNDS

Diet	Beta-glucuronidase activity (nmol/hr per mg protein)						
	Liver	Lung	Kidney	Fore-stomach	Urinary bladder	Small intestine	Colon
Control	75 ± 6[a]	37 ± 3	24 ± 2	10 ± 1	28 ± 4	39 ± 3	67 ± 4
Exptl.[b]	77-80[c]	37-39	21-23	9-10	22-29	36-46	66-68

[a] Mean ± S.E.M. (n = 6).

[b] Mice received the bisethylxanthogen, disulfiram, or diethyldithiocarbamate diet for 14 days prior to sacrifice.

[c] Organ homogenates from 6 mice in each dietary group were assayed for beta-glucuronidase activity. The range of the means for mice that received the three experimental diets is shown for each organ.

by at least two mouse liver UDP-glucuronosyltransferases [12], was induced in livers of CD-1 mice fed disulfiram, but not those fed diethyldithiocarbamate. These results do not necessarily indicate a species difference in the inducibility of 4-nitrophenol glucuronidation by diethyldithiocarbamate, since somewhat different experimental conditions were used in the two laboratories.

In diethyldithiocarbamate treated mice, none of the UDP-glucuronosyltransferase activities measured was increased significantly. The induction of these enzymes in other organs by diethyldithiocarbamate was not examined in the present investigation and can not be precluded. Previous studies showed that diethyldithiocarbamate, while exhibiting little effect on mouse liver and lung glutathione transferase activities and failing to induce liver DT-diaphorase, was a very effective inducer of DT-diaphorase and glutathione transferases in the intestinal mucosa, increasing these enzyme activities 2.6-fold to 4.8-fold [5,6]. Other effects of diethyldithiocarbamate in vivo include immunostimulation, inhibition of superoxide dismutase, inhibition of mixed function oxygenases, and inhibition of multistage tumor promotion in mouse skin [4,21,22,23].

The induction of UDP-glucuronosyltransferase activities by disulfiram and bisethylxanthogen was not accompanied by increased beta-glucuronidase activity in any of the organs examined. Glucuronidation may nevertheless be followed in some cases by transport of a glucuronide to another organ and subsequent release of a toxic or carcinogenic aglycone at a susceptible site [8,24]. Such a sequence of events may be responsible for altered organotropy of the tumorigenic effects of certain carcinogens, such as that observed in disulfiram treated animals [1]. Enhanced glucuronidation in response to the administration of disulfiram and of bisethylxanthogen may also protect against carcinogenic and toxic chemicals, since the major role of UDP-glucuronosyltransferases is to facilitate the excretion of xenobiotics, as evidenced by the large number of compounds for which glucuronides are major urinary and biliary metabolites [8].

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