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DIFFERENTIAL REGULATION OF MALE RAT LIVER GLUTATHIONE *S*-TRANSFERASES

EFFECTS OF ORCHIDECTOMY AND HORMONE REPLACEMENT

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Abstract—The effects of orchidectomy and hormone replacement on glutathione *S*-transferase activities in adult male rat liver were investigated. Due to the overlapping yet distinct substrate specificities of the hepatic glutathione *S*-transferases, we measured activity in cytosol toward four substrates: 1-chloro-2,4-dinitrobenzene, 1,2-dichloro-4-nitrobenzene, *trans*-4-phenylbut-3-en-2-one and *p*-nitrobenzyl chloride. Orchidectomy resulted in a decrease in transferase activity toward 1-chloro-2,4-dinitrobenzene, *trans*-4-phenylbut-3-en-2-one and *p*-nitrobenzyl chloride to 76, 64 and 70% of control. In contrast, transferase activity toward 1,2-dichloro-4-nitrobenzene was increased to 137% of control. To determine the role of specific androgens in the hormonal dependence of the glutathione *S*-transferases, rats were subcutaneously implanted for 4 weeks with either blank or steroid-filled sustained-release capsules at the time of orchidectomy. Transferase activities toward 1-chloro-2,4-dinitrobenzene or *p*-nitrobenzyl chloride were increased to control levels by testosterone but not by any of its 5α-reduced metabolites. Transferase activity toward *trans*-4-phenylbut-3-en-2-one was increased to control level by either dihydrotestosterone or 5α-androstan-3-α,17β-diol. Activity toward 1,2-dichloro-4-nitrobenzene was decreased to control level by all of the androgens studied, testosterone, dihydrotestosterone, 5α-androstan-3β,17β-diol or 5α-androstan-3α,17β-diol. Thus, the hepatic glutathione *S*-transferases are under separate control and are differentially regulated by testosterone and its 5α-reduced metabolites.

The glutathione *S*-transferases (EC 2.5.1.18) are a family of enzymes that catalyze the conjugation of a variety of reactive electrophiles to the nucleophile, glutathione [1, 2]. They have overlapping yet distinct substrate specificities and constitute 5–10% of the liver cytosol protein [3, 4]. Studies on the regulation of the hepatic transferases have demonstrated alterations with xenobiotic induction, age and sex [5–10]. Darby and Grundy [5] and Kaplowitz *et al.* [9] have reported sex differences in hepatic glutathione *S*-transferase activities with 1,2-dichloro-4-nitrobenzene or *p*-nitrobenzyl chloride as substrates. A previous study from our laboratory reported that the ratio of activities toward 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene in hepatic cytosol from female rats was twice that from male rats [6]. This ratio change gives an indication of alterations in the proportion of the various transferases present. Using immunoprecipitation techniques, the sex difference in activity toward these two substrates was found to reflect a difference in the amount of one specific transferase, transferase B, present.

The sexual dimorphism in glutathione *S*-transferase B concentration was eliminated by hypophysectomy [6]. More extensive studies on the hormonal regulation of the hepatic glutathione *S*-transferases using orchidectomy have yielded conflicting results. Gontovnick *et al.* [11] reported a significant decrease in hepatic glutathione *S*-trasferase activity toward styrene oxide in castrated male rats. Though Lamartiniere and Kita [12] found that castration of postpubertal rats did not alter hepatic glutathione *S*-transferase activities toward 1-chloro-2,4-dinitrobenzene or 1,2-dichloro-4-nitrobenzene, it is difficult to compare these studies as the substrates used probably reflect the activity of different members of the family of transferases. The effect of orchidectomy and of hormone replacement on hepatic glutathione *S*-transferase activity toward an array of substrates has not been studied. In this report, the hormonal dependence of transferase activity toward four substrates, 1-chloro-2,4-dinitrobenzene, 1,2-dichloro-4-nitrobenzene, *trans*-4-phenylbut-3-en-2-one and *p*-nitrobenzyl chloride, is investigated following orchidectomy and immediate hormone replacement with testosterone or its 5α-reduced metabolites, dihydrotestosterone, 5α-androstan-3β,17β-diol or 5α-androstan-3α,17β-diol. Orchidectomy resulted in a decrease in transferase activity toward three of these substrates and an increase in transferase activity toward the fourth. For each transferase substrate measured, the four androgens administered differed in their abilities to reverse the effects of orchidectomy. These changes in transferase activity toward specific substrates are related to the individual transferases.

MATERIALS AND METHODS

Chemicals. 1-Chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene were obtained from the Eastman Kodak Co., Rochester, NY, and *trans*-4-phenylbut-3-en-2-one from the Aldrich Chemical Co., Milwaukee, WI. *p*-Nitrobenzyl chloride was

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obtained from ICN-K & K Laboratories, Plainview, NY. Bovine serum albumin and glutathione were obtained from the Sigma Chemical Co., St. Louis, MO. Polydimethylsiloxane (PDS, Silastic) tubing (no. 602-305) and adhesive (no. 891) were obtained from the Dow Corning Co., Midland, MI. Testosterone, dihydrotestosterone, 5α-androstan-3β,17β-diol and 5α-androstan-3α,17β-diol were purchased from Steraloids, Wilton, NH.

Animals and tissue preparation. Adult male Sprague–Dawley rats (250–300 g), obtained from Charles River Canada Inc. (St. Constant, Quebec), were housed on Beta-chips (Charles River Canada Inc.), at the Royal Victoria Hospital and maintained on a 14 hr light/10 hr dark cycle. They received water and Purina rat chow *ad lib.* and were assigned to one of ten treatment groups. The control group received blank (empty) subdermal PDS implants. The remaining nine groups were castrated under ether anesthesia and simultaneously implanted either with empty capsules or with testosterone (0.5 or 2.5 cm), dihydrotestosterone (0.7 or 3.3 cm), 5α-androstan-3β,17β-diol (1.6 or 8.0 cm) or 5α-androstan-3α,17β-diol (1.6 or 8.0 cm) filled PDS capsules (prepared according to the method of Stratton *et al.* [13]). The high dose of testosterone (relative dose 1.0) was selected as the dose that maintains sex accessory tissue weights (ventral prostrate and seminal vesicles) and serum levels of testosterone in a range similar to that of control uncastrated rats (data not shown). The release rates for testosterone, dihydrotestosterone and 5α-androstan-3α,17β-diol are approximately 30 μg per day per cm [14], 24 and 8 μg per day per cm (C. Desjardins, personal communication) respectively. The lengths of the implants of the three 5α-reduced androgens were adjusted so that daily hormone release rates similar to that of testosterone would be obtained.

Rats were decapitated 4 weeks after orchidectomy. The excised livers were frozen in liquid nitrogen and stored at −80°. For preparation of the cytosol fraction, the livers were thawed and homogenized (1:3, w/v) in ice-cold 10 mM potassium phosphate buffer, pH 7.0, containing 20% (v/v) glycerol with a Potter–Elvehjem homogenizer. Homogenates were centrifuged at 105,000 *g* for 60 min and the supernatant fraction was assayed for glutathione *S*-transferase activity and protein content. Freezing caused approximately a 30% decrease in enzyme activity. This decrease was identical for transferase activity toward each of the substrates measured and was not altered by the length of storage at −80° up to 1 month.

Glutathione S-transferase assays. Assay conditions for measurement of the conjugation of 1-chloro-2,4-dinitrobenzene, 1,2-dichloro-4-nitrobenzene, *trans*-4-phenylbut-3-en-2-one and *p*-nitrobenzyl chloride with glutathione were identical with those of Habig *et al.* [15]. Assays were done at room temperature (21–23°) and were followed with a Beckman model 35 recording spectrophotometer. Raction rates were linear with protein concentration and with time for at least 2 min.

Protein concentrations were measured by the method of Lowry *et al.* [16], with bovine serum albumin as a standard.

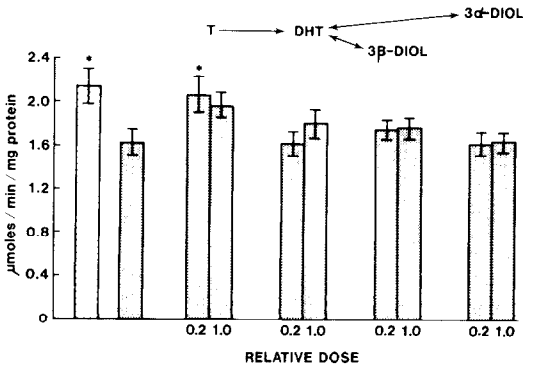

Fig. 1. Effect of orchidectomy and androgen replacement on hepatic glutathione *S*-transferase activity toward 1-chloro-2,4-dinitrobenzene. Control rats (□) received empty implants. Orchidectomized rats (□) received subcutaneous blank, testosterone (T, 0.5 or 2.5 cm, relative dose 0.2 and 1.0, respectively), dihydrotestosterone (DHT, 0.7 or 3.3 cm, relative dose 0.2 and 1.0, respectively), 5α-androstan-3β,17β-diol (3β-diol, 1.6 or 8.0 cm, relative dose 0.2 and 1.0, respectively), or 5α-androstan-3α-17β-diol (3α-diol, 1.6 or 8.0 cm, relative dose 0.2 and 1.0, respectively) filled polydimethylsiloxane implants at the time of surgery. Enzyme activity was assayed as described in the text. Values are means ± S.E.M. for cytosol preparations from six animals. Treatment groups marked with an asterisk (*) differed significantly from the orchidectomized group, $P \leq 0.05$, Duncan's multiple range test.

Statistical evaluation. Data were analyzed by one-way analysis of variance followed by Duncan's multiple range test [17, 18].

RESULTS

The effects of orchidectomy and androgen replacement on hepatic glutathione *S*-transferase activity toward 1-chloro-2,4-dinitrobenzene are shown in Fig. 1. Orchidectomy alone caused a decrease in transferase activity to 76% of control ($P < 0.05$).

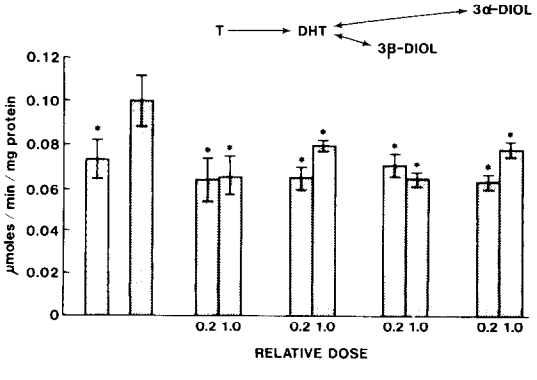

Fig. 2. Effect of orchidectomy and hormone replacement on hepatic glutathione *S*-transferase activity toward 1,2-dichloro-4-nitrobenzene. Control rats (□) and orchidectomized rats (□) received implants as described in the text and the legend to Fig. 1. Values are means ± S.E.M. ($N = 6$). Treatment groups marked with an asterisk (*) differed significantly ($P \leq 0.05$) from the orchidectomized group.

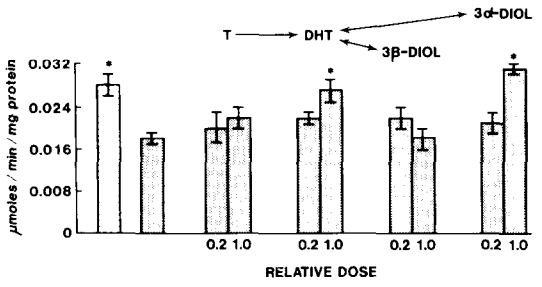

Fig. 3. Effect of orchidectomy and hormone replacement on hepatic glutathione S-transferase activity toward *trans*-4-phenylbut-3-en-2-one. Control rats (□) and orchidectomized rats (▨) received implants as described in the text and the legend to Fig. 1. Values are means ± S.E.M. (N = 6). Treatment groups marked with an asterisk (*) differed signficantly ($P \leq 0.05$) from the orchidectomized group.

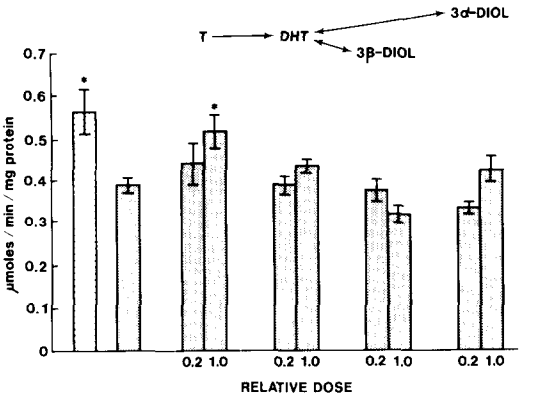

Fig. 4. Effect of orchidectomy and hormone replacement on hepatic glutathione S-transferase activity toward *p*-nitrobenzyl chloride. Control rats (□) and orchidectomized rats (▨) received implants as described in the text and the legend to Fig. 1. Values are means ± S.E.M. (N = 6). Treatment groups marked with an asterisk (*) differed significantly ($P \leq 0.05$) from the orchidectomized group.

The low dose of testosterone was sufficient to maintain this activity at the control value. No further change in activity was found by raising the testosterone dose 5-fold. None of the three 5α-reduced androgens tested altered the transferase activity from that of castrate. Hence, the effect of castration on transferase activity toward this substrate was countered only by testosterone.

The effects of orchidectomy and hormone replacement on transferase activity toward 1,2-dichloro-4-nitrobenzene were in marked contrast to those on activity to 1-chloro-2,4-dinitrobenzene (Fig. 2). A significant ($P < 0.05$) increase of nearly 40% in activity toward this substrate was noted. All four androgens tested had a similar effect, i.e. a reversal of the castration effect, decreasing the activity down to the control level. No significant differences with respect to this enzymatic activity were found between any of the eight androgen-treated groups ($P > 0.05$).

The activity of the glutathione S-transferases toward *trans*-4-phenylbut-3-en-2-one was reduced to less than two-thirds of the control after orchidectomy (Fig. 3). Neither testosterone nor 5-α-androstan-3β,17β-diol caused any significant alteration in transferase activity when compared to castrate; the other two 5α-reduced androgens, dihydrotestosterone and 5α-androstan-3α-17β-diol, maintained this activity at the control level, but only with the higher dose used.

The pattern of hormonal response of glutathione S-transferase activity toward *p*-nitrobenzyl chloride (Fig. 4) was nearly identical to that seen for activity toward 1-chloro-2,4-dinitrobenzene. A decrease to 70% of control was observed after orchidectomy. This decrease was reversed selectively by testosterone. However, with this substrate, a high dose of testosterone was necessary to maintain transferase activity at the control level.

Ratios of the glutathione S-transferase activities toward two substrates give an indication of changes in the relative proportion of individual forms of this family of enzymes. Such ratios for the effect of orchidectomy and hormone replacement are pre-

Table 1. Effect of orchidectomy and androgen replacement on the ratios of activities of the hepatic glutathione S-transferases

			ORC and androgen replacement*							
			T		DHT		3β-diol		3α-diol	
Ratio of activities	Control	ORC	0.2	1.0	0.2	1.0	0.2	1.0	0.2	1.0
CDNB/DCNB†	29	16	33	31	26	23	25	28	26	21
NBC/TPBO‡	20	22	22	24	18	16	17	18	16	14

* Control rats received empty implants. Orchidectomized rats received subcutaneous blank (ORC), testosterone (T, 0.5 or 2.5 cm, relative dose 0.2 and 1.0, respectively), dihydrotestosterone (DHT, 0.7 or 3.3 cm, relative dose 0.2 and 1.0, respectively), 5α-androstan-3β,17β-diol (3β-diol, 1.6 or 8.0 cm, relative dose 0.2 and 1.0, respectively), or 5α-androstan-3α,17β-diol (3α-diol, 1.6 or 8.0 cm, relative dose 0.2 and 1.0, respectively) filled polydimethylsiloxane implants at the time of surgery. Enzyme activity was assayed as described in the text. Values are means of the ratios of activities toward two substrates.

† CDNB/DCNB designates the ratio of activity toward 1-chloro-2,4-dinitrobenzene to that toward 1,2-dichloro-4-nitrobenzene.

‡ NBC/TPBO designates the ratio of activity toward *p*-nitrobenzyl chloride to that toward *trans*-4-phenylbut-3-en-2-one.

Table 2. Specific activities of the purified rat glutathione *S*-transferases*

Substrate	Transferase			
	A	B	C	E
1-Chloro-2,4-dinitrobenzene	62	11	10	0.01
1,2-Dichloro-4-nitrobenzene	4.3	0.003	2.0	0
trans-4-Phenylbut-3-en-2-one	0.02	0.001	0.40	0
p-Nitrobenzyl chloride	11.4	0.1	10.2	4.1

* Data taken from Ref. 3, are expressed in μmoles per min per mg protein.

sented in Table 1. Orchidectomy decreased the ratio of activity toward 1-chloro-2,4-dinitrobenzene to 1,2-dichloro-4-nitrobenzene. Testosterone administration maintained this ratio at or above the control value by maintaining activity toward both 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene at control levels. All three of the 5α-reduced androgens tested increased this ratio above the orchidectomy value by decreasing activity toward 1,2-dichloro-4-nitrobenzene to the control level.

In contrast, orchidectomy itself had no significant effect on the ratio of transferase activity toward *p*-nitrobenzyl chloride to *trans*-4-phenylbut-3-en-2-one (activity toward both substrates decreased proportionally). This ratio was not changed by high dose testosterone treatment and decreased by treatment will all three of the 5α-reduced androgens.

DISCUSSION

Orchidectomy resulted in a decrease in hepatic glutathione *S*-transferase activity toward 1-chloro-2,4-dinitrobenzene, *trans*-4-phenylbut-3-en-2-one and *p*-nitrobenzyl chloride but in an increase in activity toward 1,2-dichloro-4-nitrobenzene. Previous studies have shown that male rat liver has three times more transferase activity toward 1,2-dichloro-4-nitrobenzene than female rat liver [6]. Thus, the changes in hepatic transferase activity after gonadectomy of the male do not represent a conversion of the male to the female pattern of activity.

Experiments on the effect of orchidectomy followed by immedate hormone replacement on the hepatic glutathione *S*-transferase activity toward these four substrates provided further information on the regulation of this family of enzymes. Testosterone was effective in maintaining transferase activity toward 1-chloro-2,4-dinitrobenzene, 1,2-dichloro-4-nitrobenzene and *p*-nitrobenzyl chloride but not *trans*-4-phenylbut-3-en-2-one at the control level. In contrast, two of the 5α-reduced androgens, dihydrotestosterone and 5α-androstan-3α,17β-diol, maintained activity toward *trans*-4-phenylbut-3-en-2-one; they also maintained activity toward 1,2-dichloro-4-nitrobenzene but could not maintain transferase activity toward the other two substrates maintained by testosterone. 5α-Androstan-3β,17β-diol was unable to maintain activity toward all but one substrate, 1,2-dichloro-4-nitrobenzene. The differential control by the aromatizable androgen, testosterone, and the non-aromatizable 5α-reduced androgens suggests that some of the transferases may be regulated by estrogens, others by 5α-reduced androgens, whilst others still may be controlled by any androgen or potentially any steroid. This selectivity in androgen action is consistent with numerous other studies where these androgens have been shown to have differential biological activities [19–21].

Since the hepatic glutathione *S*-transferases have overlapping substrate specificities [3] (Table 2) and the same molecular weights, it is possible to resolve these proteins only by their differences in isoelectric point [4, 22]. Although transferase activity toward these substrates reflects the cumulative activity of two or more of these proteins, changes in the ratios of activities toward specific substrates have been used previously to predict changes in the proportion of one of the transferases present [6, 22]. Based on the substrate specificities of the purified glutathione *S*-transferases reported by Jakoby *et al.* [3], we interpret our results to indicate an increase in the activity of transferase A and a decrease in the other transferases after orchidectomy. The ratio of activities toward 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene decreased after orchidectomy (Table 1) which indicates a decrease in the relative proportion of transferases B or AA and an increase in transferases A or C. The possibility of an increase in transferase C after orchidectomy is eliminated by the maintenance of the ratio of activities of *p*-nitrobenzyl chloride to *trans*-4-phenylbut-3-en-2-one in orchidectomized animals. Using this approach, it may be concluded that all the androgens administered can maintain transferase A activity at the control level, whereas only the 5α-reduced androgens can also maintain the activity of transferase C.

It is thus apparent that hepatic glutathione *S*-transferase activities toward these four substrates are differentially regulated by testosterone and its 5-α-reduced metabolites.

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