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Biochemical Pharmacology, Vol. 31, No. 23, pp. 3927–3930, 1982.
Printed in Great Britain.

0006-2952/82/233927-04 $03.00/0

Regulation of brain and hepatic glutathione-*S*-transferase by sex hormones in rats

(*Received* 23 *February* 1982; *accepted* 7 *July* 1982)

The presence of glutathione-*S*-transferase (GST)* activity in mammalian and avian brains and its characterization in rat brain has been reported by us recently [1, 2]. Brain and hepatic GSTs were found to catalyze the conjugation of acrylamide with GSH [3] and to bind this potent neurotoxin [4]. The enzyme may therefore play a biological role in removal and expression of the toxicity of certain neurotoxic xenobiotics.

The brain regulates the secretion of hormones in pre- and post-pubertal animals. Recent studies by Lamartiniere [5] have shown that the hypothalamic–hypophyseal–gonadal axis plays an important role in the sexual differentiation and regulates the hepatic GST activity in the rat. Evidence has been presented to show that the hypothalamic nerve endings of female rats exposed to androgen during the critical period of early development result in the programming of a male type of metabolism that is expressed post-pubertally via the hypothalamic–hypophyseal–gonadal axis [6–10]. Therefore it would be of potential interest to investigate whether brain GST is regulated by these sex hormones. The present study deals with some of our observations on the regulation of brain and hepatic GSTs by sex hormones.

Material and methods

The experiments were performed on young and post-pubertal Wistar albino rats derived from the ITRC animal breeding colony. The animals were raised on a commercial pellet diet (Hindustan Lever, Bombay, India) and allowed free access to water. The litters were kept with their mothers until weaning, i.e. until 3 weeks of age (five pups with one mother per cage). After weaning five animals were housed in a cage.

Post-pubertal male rats (12 weeks old) were castrated by removing both the testicles after anaesthetising the animals with ether. Anaesthetic ether was found to produce no significant change in GST activity of the brain and liver. Castration or sham operations in neonatal (1-day-old) male rats were performed by placing them in an ice-water bath prior to surgery.

TP and DES [each from Steriod Inc. (Wilton, NH)] were dissolved in peanut oil and injected at a dose of 2.5 mg/kg body weight subcutaneously in the adult rats daily for 7 days beginning 1 week after castration. The control rats received the same volume of vehicle. All the animals were killed 7 days after the last treatment. In the case of neonatal rats the pups were given TP and/or DES from day 7 to 13 of their life and killed by decapitation at 3 and 9 weeks of age.

The brain and liver homogenates were centrifuged at 14,000 and 9000 *g* for 15 min respectively to obtain post-mitochondrial fractions which were used for the measurement of GST activity by the method of Habig *et al.* [11].

GSH and protein contents were estimated according to the methods of Ellman [12] and Lowry *et al.* [13] respectively.

Results

Effect of neonatal castration and influence of sex hormones on brain and hepatic GST activity in pre- and post-pubertal rats.

At prepubertal (3 weeks) age, brain and hepatic GST activity towards CDNB did not show any significant difference between male and female rats (Fig. 1 and Table 1). The GST activity in both tissues remained unaffected on neonatal castration at prepubertal age but administration of TP and DES to castrated pups induced enzyme activity significant in comparison to uncastrated male or female animals (Fig. 1 and Table 1). No change in brain or hepatic GSH content was observed on castration or treatment of castrated rats with TP or DES (data not shown).

Fig. 2 demonstrates that brain GST activity towards CDNB of male rats at post-pubertal age (9 weeks) was significantly lower than that of females. Neonatal castration

* Abbreviations: GST, glutathione-*S*-transferase; GSH, glutathione; TP, testosterone propionate; DES, diethystilbestrol; CDNB, 1-chloro-2,4-dinitrobenzene.

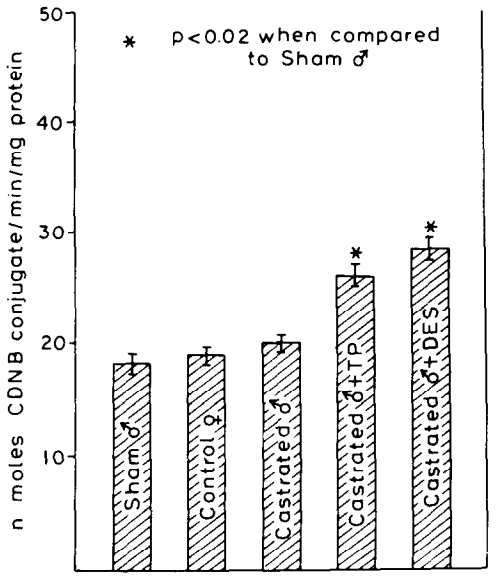

Fig. 1. Effect of neonatal castration on brain glutathione-*S*-transferase activity in prepubertal rats (3 weeks). Castration and treatment of animals were performed as described in the text. *P < 0.02, when compared to sham operated males.

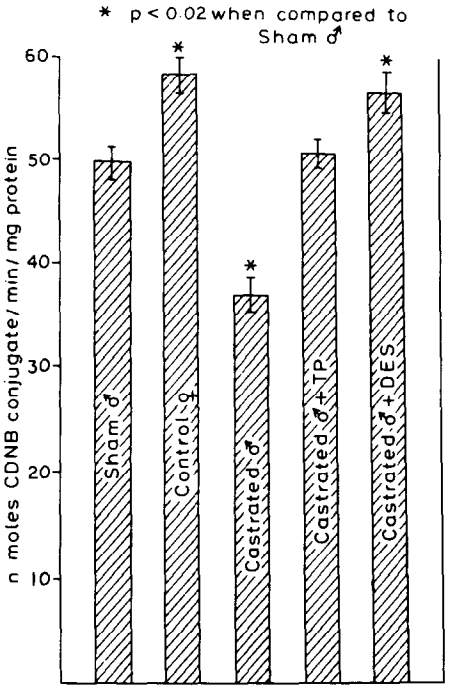

Fig. 2. Effect of neonatal castration on brain glutathione-*S*-transferase activity in post-pubertal rats (9 weeks): castration and treatment of animals were performed as described in the text. *P < 0.02, when compared to sham operated males.

caused a significant decrease in GST activity in comparison to normal male rats at this age. Supplementation of TP and DES to castrated rats restored the enzyme activity to levels equal to those of male and female rats (Fig. 2). Hepatic GST activity towards CDNB in male rats at post-pubertal age (9 weeks) was significantly higher than in females (Table 1). Castration of male animals at birth caused a significant decrease in hepatic GST activity towards CDNB at post-pubertal age (9 weeks). Administration of TP and DES to castrated rats restored the levels of the enzyme activity to those of control males and females (Table 1). No change in brain and hepatic glutathione content was observed on castration or treatment of castrated rats with TP and DES (data not shown).

Effect of castration on post-pubertal (12 weeks) rats and influence of sex hormones on brain and hepatic GST activity. As observed earlier, brain GST activity towards CDNB was significantly higher in post-pubertal female rats in comparison to post-pubertal males. Post-pubertal castration caused a significant decrease in brain GST activity towards CDNB in male rats while administration of TP and DES to castrated rats induced enzyme levels equivalent to that of normal male and normal female rats (Fig. 3). Hepatic GST activity towards CDNB exhibited a lower value in females than males. Unlike the neonatal castration, post-pubertal castration caused a decrease in hepatic GST activity towards CDNB in male rats. Administration of TP to the castrated male rats induced hepatic GST activity towards CDNB significantly even more than for the control males (Table 1). DES treatment to castrated male rats also induced hepatic GST activity at levels greater than normal females (Table 1). No change in brain or hepatic GSH content was also observed on post-pubertal castration of treatment of castrated rats with TP and DES (data not shown).

Table 1. Effect of neonatal castration or post-pubertal castration and influence of sex hormones on hepatic glutathione-*S*-transferase (GST) activity

Animals	Neonatal castration GST at pre-pubertal age (3 weeks)	Neonatal castration GST at post-pubertal age (9 weeks)	Post-pubertal castration GST at post-pubertal age (12 weeks)
Control sham males	275 ± 9	567 ± 11	1058 ± 40
Control females	283 ± 12	506 ± 9*	840 ± 34*
Castrated males	282 ± 10	493 ± 13*	922 ± 32*
Castrated males + TP	342 ± 11*	570 ± 14	1264 + 52*
Castrated males + DES	345 ± 13*	529 ± 11*	1123 ± 36

Data represent means ± S.E. of five values.
Castration and treatment of animals were performed as described in the text.
* P < 0.05, when compared to sham males.

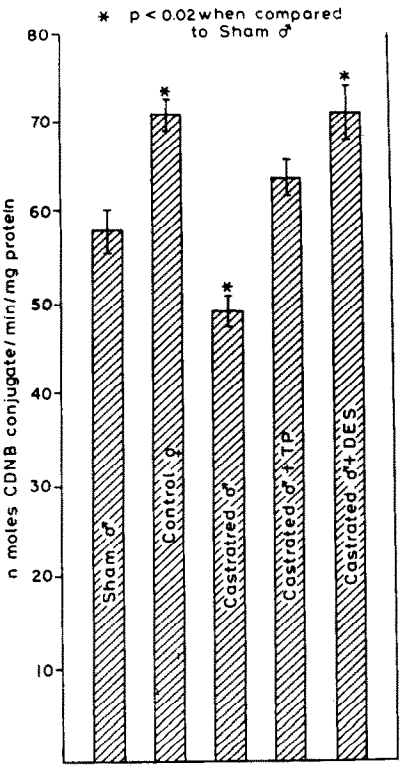

Fig. 3. Effect of post-pubertal castration on brain glutathione-*S*-transferase activity in adult rats (12 weeks): castration and treatment of animals were performed as described in the text. *P < 0.02, when compared to sham operated males.

Discussion

Numerous studies have been conducted on the neonatal development of GST activity in the liver and other tissues [14–16]. Hepatic GST activity has been shown to increase until 15 weeks of age with styrene oxide as substrate [14, 15]. A developmental pattern of hepatic GST activity towards CDNB has been seen only until 6 or 7 weeks of age and an increase until that time has been observed [14, 15]. In the present study we have observed an increase in hepatic and brain GST until 12 weeks of age. These observations were consistent with our previous findings [2] and the studies of Lamartiniere [5]. Neonatally, sex-related differences in the enzyme activity were not encountered in both the tissues while at post-pubertal age a sex-related difference in GST activity in the brain as well as in the liver was evident.

The finding of no significant change in brain and hepatic GST activity in neonatally castrated pups at prepubertal age (3 weeks) may be due to the low levels of hormones [17, 18]. Induction of GST activity in the brain and liver on administration of TP or DES to castrated pups demonstrated the influence of hormones on enzyme activity. The decrease in hepatic and brain GST activity at post-pubertal age (9 weeks) of rats castrated neonatally (1 day of age) and restoration of the enzyme activity by TP and DES in neonatally castrated pups further indicates the involvement of sex hormones.

A sex-dependent difference in hepatic and brain GST activity was also evident in adult rats of 12 weeks of age. Lamartiniere [5] has also observed a sex-related difference in hepatic GST activity at this age. A significant decrease in GST activity on removal of the testis at post-pubertal age and induction of the enzyme activity by supplementation of TP in both tissues observed in this study further demonstrates the influence of sex hormones on GST activity. Female sex hormone also induced the enzyme activity in post-pubertal rats. However, no such effect of castration or exogenous sex hormones was observed by Lamartiniere [5] and Reyes *et al.* [19]. This difference may be due to the variations in the animal species.

No change in the levels of GSH with respect to castration or supplementation of sex hormones suggests that only brain and hepatic GST protein is influenced by sex hormones and not its conjugating factor (GSH).

Summary

Our results indicate that sex hormones play an important role in the regulation of brain and hepatic GST protein during maturity. The conjugating factor GSH does not appear to be under the influence of sex hormones. These observations are of great significance in view of the possibility of continued exposure to neurotoxic chemicals like DDT and Kepone which can cause significant alterations in levels of sex hormones. A reduced GSH activity could lead to a retarded biotransformation of electrophiles and thus to an enhanced toxicity.

Acknowledgements—We are indebted to Dr C. R. Krishna Murti, Director, Industrial Toxicology Research Centre, Lucknow, India, for his keen interest in the present study. M. Das is thankful to CSIR, New Delhi, India, for the award of a Senior Research Fellowship.

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Biochemical Pharmacology, Vol. 31, No. 23, pp. 3930–3933, 1982.
Printed in Great Britain.

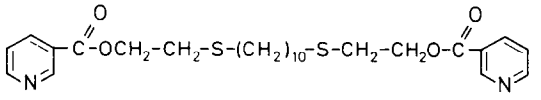
0006-2952/82/233930-04 $03.00/0

Lipid-metabolizing enzymes, CoASH and long-chain acyl-CoA in rat liver after treatment with tiadenol, nicotinic acid and niadenate

(*Received* 25 *March* 1982; *accepted* 23 *July* 1982)

Tiadenol [bis(hydroxy-ethylthio)-1,10-decane] is highly effective in lowering the serum concentration of cholesterol, triglycerides and the VLDL + LDL/HDL ratio in different types of hyperlipoproteinemias [1–3]. However, the drug is also more potent that the hitherto most widely used hypolipidemic drug, clofibrate, in inducing hepatomegaly associated with peroxisomal proliferation and increased activity of some hepatic lipid-metabolizing enzymes in the rat [4–7]. A number of hypolipidemic drugs which induce hepatomegaly and peroxisomal proliferation have been shown to cause liver tumors in rats and mice [5, 8].

Nicotinic acid represents a different class of hypolipidemic drugs and is thought to act mainly by inhibiting peripheral lipolysis thereby making less free fatty acids available for the synthesis of triglycerides, VLDL and eventually LDL [9–11]. Niadenate is a prodrug of nicotinic acid and tiadenol:



The hydrolysis of the ester thus yields two active compounds with distinct mechanisms of action. Since marked increase and subcellular redistribution of peroxisomal enzyme activities occur after clofibrate or tiadenol treatment [6, 12] we have carried out a study with niadenate and nicotinic acid including tiadenol as a reference drug.

Materials and methods. Male Wistar rats weighing 250–270 g were randomly selected for drug treatment or for control experiments. Tiadenol, nicotinic acid or niadenate were added to the diet by soaking commercially available rat food with an acetone solution as described previously [6] leaving pellets containing 0.3, 0.25 and 0.5% (w/w), respectively, of the drugs. The concentration of tiadenol and niadenate in the diets was equimolar whereas that of nicotinic acid matched the amount produced by hydrolysis of niadenate (two molecules of nicotinic acid per molecule of the prodrug). All drugs were obtained from Laboratorios Almirall S.A. (Barcelona, Spain).

Pairs of animals were housed in separate cages and fed the experimental diets with water *ad lib.* for 10 days. The food intake was measured daily for each pair of rats to get an estimate of the average daily drug dose. The rats were killed by decapitation and the livers were homogenized.

The homogenates were fractionated into nuclear (N), mitochondrial (M), light-mitochondrial (L), microsomal (P) and particle-free supernatant (S) fractions according to DeDuve *et al.* [13] with a few modifications [12]. All procedures were performed at 0–4°, and the fractions were stored below −20° until analyzed. The enzyme assays were carried out as described elsewhere [12]. CoASH and long-chain CoA were determined using a published high-pressure liquid chromatographic method [14] with some modifications [12].

Results and discussion. The liver weight increased considerably in the tiadenol- and niadenate-treated animals, and the hepatomegaly was accompanied by a marked increase in protein content (Table 1). The drugs caused little change in the DNA content per gram liver, but there was a trend towards lower relative amounts (μg/mg protein) in the tiadenol-treated animals. Like the liver enlargement produced by clofibrate and some other lipid-lowering drugs [5, 15] the findings of the present study may be interpreted as indicating both an increase in cell size and an increased number of hepatocytes.

Both tiadenol and niadenate elicited a large increase in the amount of long-chain acyl-CoA and free CoASH whereas nicotinic acid influenced the levels to a much smaller extent (Table 1). The concentration of free CoASH per gram liver increased almost 10-fold after niadenate feeding. The total amounts as well as the activities per gram liver of palmitoyl-CoA hydrolase went up several-fold after treatment with tiadenol or niadenate (Table 2). The increase in the palmitoyl-L-carnitine hydrolase and sedimentable palmitoyl-CoA hydrolase activities was less pronounced. A 10–20-fold increase was observed in peroxisomal palmitoyl oxidation after tiadenol or niadenate administration. Total catalase was increased after treatment with tiadenol or niadenate, but the sedimentable enzyme activity was almost unchanged. The activity of urate oxidase per gram liver was not elevated after drug treatment, and there was rather a trend toward lower values in the tiadenol and niadenate experiments.

The increase in palmitoyl-CoA hydrolase activity after tiadenol or niadenate treatment occurred mainly in the M- and S-fractions with a concomitant reduction in the P-fraction (Fig. 1). The relative distribution of sedimentable palmitoyl-CoA hydrolase was less affected. Palmitoyl-L-carnitine hydrolase increased mainly in the supernatant S-fraction at the expense of the P-fraction which normally contains the highest activities.

A large increase in peroxisomal palmitoyl-CoA oxidation was observed in the supernatant after tiadenol or niadenate feeding. A marked reduction occurred in the L-fraction resulting in higher percentages of sedimentable activity in the heaviest fractions (N and M). A redistribution of catalase activity was observed with higher amounts in the supernatant and a lower relative sp. act. in the L-fraction after tiadenol or niadenate administration. These drugs also elicited a redistribution of urate oxidase from the L-