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SEX DIFFERENCES IN BIOCHEMICAL MANIFESTATIONS OF SELENIUM DEFICIENCY IN RAT LIVER WITH SPECIAL REFERENCE TO HEME METABOLISM

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Abstract—In addition to its well-known effect on glutathione peroxidase, selenium deficiency causes: (1) a defect in hepatic heme metabolism characterized by a phenobarbital-mediated increase in microsomal heme oxygenase activity, and (2) an increase in hepatic glutathione *S*-transferase activity. Since these effects were reported in selenium-deficient male rats, and since female rats have a lower selenium requirement than males, we examined whether these effects were sex-dependent. Weanling male rats, female rats, castrated male rats, and testosterone-treated female rats were fed either a selenium-deficient or a control diet. After 8 weeks, selenium-dependent hepatic glutathione peroxidase activity was 1 per cent of respective control values in each of the selenium-deficient groups. Hepatic glutathione *S*-transferase activity was doubled by selenium deficiency in normal, unoperated males but was unaffected in the other groups. In control diet fed rats phenobarbital given as a single injection caused either no significant change or a decrease in the activity of hepatic microsomal heme oxygenase, the rate-limiting enzyme in heme degradation. In contrast, microsomal heme oxygenase activity was stimulated by phenobarbital in all selenium-deficient rats. The stimulation was greatest in males and least in females with intermediate values in castrated males and testosterone-treated females. These results demonstrate a marked effect of sex, castration of males, and testosterone treatment of females on the response of hepatic heme metabolism to phenobarbital and on glutathione *S*-transferase activity in selenium deficiency even though glutathione peroxidase was reduced to the same extent by selenium deficiency in all groups.

Recently, some changes in hepatic enzymes other than the well-known decrease in glutathione peroxidase activity [1] have been reported in selenium deficiency. We have demonstrated that a defect in heme metabolism occurs in the selenium-deficient rat liver which, under some conditions, results in increased synthesis and degradation of heme along with decreased heme availability for formation of hemoproteins such as cytochrome P-450 [2, 3]. The primary biochemical manifestation of this defect is a rapid stimulation by phenobarbital of the hepatic activity of microsomal heme oxygenase, the rate-limiting enzyme in the heme catabolic pathway [4]. No such stimulation occurs in selenium-adequate rat liver.

Another hepatic enzyme alteration found in selenium deficiency is an increase in glutathione *S*-transferase activity [5]. The glutathione *S*-transferases, comprising 5–10 per cent of the cytosolic protein, catalyze the conjugation of glutathione with a wide variety of compounds, detoxifying many of them [6]. They also bind many compounds which are not substrates, including bilirubin, and, it has been postulated, may serve as temporary storage sites for them [7]. Some of these enzymes can also function as glutathione peroxidases [5]. These findings have implications for toxicity studies which employ selenium-deficient rats as an "antioxidant-deficient" group.

Most studies of selenium deficiency have been conducted using male rats. However, since females are more resistant to developing selenium deficiency [8], and since in preliminary studies we had noted milder abnormalities of heme metabolism in female selenium-deficient rats that in males, we examined the altered hepatic activities of the enzymes of heme metabolism elicited by selenium deficiency in relation to sex. Our aims were to compare the biochemical manifestations of selenium deficiency in male and female rats and to determine the effects of castration of males and of testosterone treatment of females.

METHODS

Animals. Weanling rats, 3 weeks of age, purchased from the Holtzman Co. (Madison, WI) were fed the Torula yeast-based diet described previously [9] and given tap water *ad lib.* The diet was supplemented with 100 I.U. of vitamin E/kg as *dl*-α-tocopheryl acetate and 0.3% *dl*-methionine. Control and selenium-deficient diets were identical except for the addition of 0.5 mg selenium/kg as Na_2SeO_3 to the control diet.

One week after receipt, ten selenium-deficient and ten control male rats were castrated under light ether anesthesia. Thereafter, they were caged separately from the unoperated males. At the same time, testosterone injections were begun in ten selenium-deficient and ten control female rats which were

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caged separately from the uninjected female rats. Each rat received 2 mg testosterone subcutaneously 5 days a week for the next 7 weeks until it was killed. The testosterone injection solution was composed of 1 g testosterone in 100 ml triolein and 5 ml benzyl benzoate.

Experimental. Rats were studied 8 (Fig. 3 and Table 1) or 12 (Figs. 1 and 2) weeks after the experimental diets were begun and 7 weeks after castration or initiation of testosterone treatment. Phenobarbital (80 mg phenobarbital sodium/kg) was given intraperitoneally in 0.15 M NaCl 6 hr before death. Animals were fasted for 24 hr and weighed before being killed.

Rats were stunned by a blow to the neck and exsanguinated. Livers were perfused with 1.15% KCl, and 20% (w/v) homogenates were prepared with a motor-driven Teflon pestle and glass homogenizing vessel. The microsomal fraction was prepared as described previously [2]. The 18,000 *g* supernatant fraction was obtained by centrifuging the homogenate for 10 min, and the 105,000 *g* supernatant fraction was prepared by recentrifuging the 18,000 *g* supernatant fraction for 60 min.

Assays. δ-Aminolevulinic acid synthetase was assayed as described previously [3]. Microsomal heme oxygenase activity was measured in the 18,000 *g* supernatant fraction [3], and microsomal cytochrome P-450 was determined by the method of Raj and Estabrook [10] using a DW-2 UV-VIS spectrophotometer (American Instrument Co., Silver Springs, MD). Glutathione peroxidase activity of the 105,000 *g* liver supernatant fraction was measured with 0.25 mM H_2O_2 as substrate using the coupled assay previously employed [11]. Glutathione *S*-transferase activity was also measured in the 105,000 *g* liver supernatant fraction with 1-chloro-2,4-dinitrobenzene (1-CDNB) and 1,2-dichloro-4-nitrobenzene (1,2-DCNB) as substrates, using conditions described by Habig *et al.* [12]. Protein was determined by the method of Lowry *et al.* [13]. Student's unpaired *t*-test was used for statistical analysis.

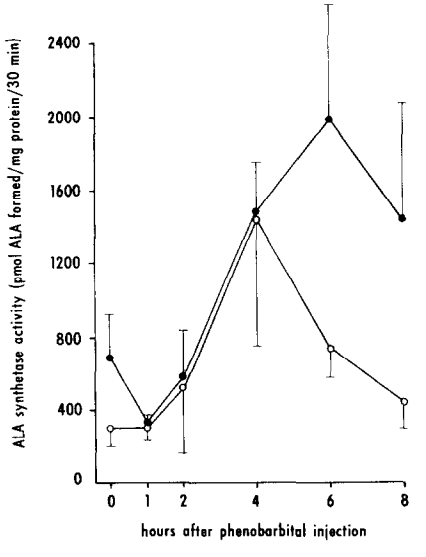

Fig. 1. Hepatic δ-aminolevulinic acid (ALA) synthetase activity after phenobarbital administration to selenium-deficient (●——●) and control (○——○) female rats. Each point represents the mean of three animals except for the 1 and 8 hr points of the control group which are the means of two animals. The half brackets are 1 S.D

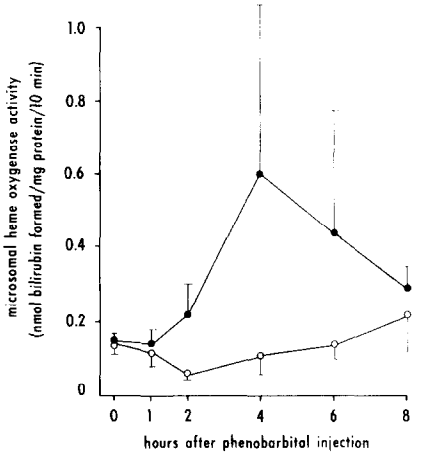

Fig. 2. Hepatic microsomal heme oxygenase activity after phenobarbital administration to selenium-deficient (●——●) and control (○——○) female rats. Each point represents the mean of three to five animals and the half brackets are 1 S.D.

Materials. Sodium phenobarbital was purchased from the Mallinckrodt Chemical Works, St. Louis, MO. Glutathione, glutathione reductase (yeast type III), EDTA and NADPH were from the Sigma Chemical Co., St. Louis, MO; 1-CDNB and 1,2-DCNB were from Eastman Organic Chemicals, Rochester, NY; and H_2O_2 was from the J. T. Baker Co., Phillipsburg, NJ. Diet constituents were purchased from ICN Pharmaceuticals, Inc., Cleveland, OH, except for Torula yeast which came from St. Regis, Rhinelander, WI.

RESULTS

δ-Aminolevulinic acid synthetase activity in female rat liver rose in response to phenobarbital (Fig. 1). There was a greater and more prolonged increase in selenium-deficient rats than in controls. Hepatic microsomal heme oxygenase activity increased in selenium-deficient female rats after phenobarbital treatment but not in controls (Fig. 2). Maximal activity was reached 4–6 hr after phenobarbital administration. These results are similar to those obtained previously with male rats [3] except that the magnitude of the increase in heme oxygenase activity was less in female rats. Since, in both male and female selenium-deficient rats, hepatic microsomal heme oxygenase activities had been stimulated 6 hr after phenobarbital, this time was chosen to study the effects of castration of males and of testosterone treatment of females. Six hr after phenobarbital administration there was a small decrease in microsomal heme oxygenase activity in all rats fed the control diet, regardless of sex or whether they were castrated or testosterone-treated (Fig. 3). In contrast, the activity rose in all selenium-deficient rats after phenobarbital, with the greatest increase observed in the noncastrated males and the least in

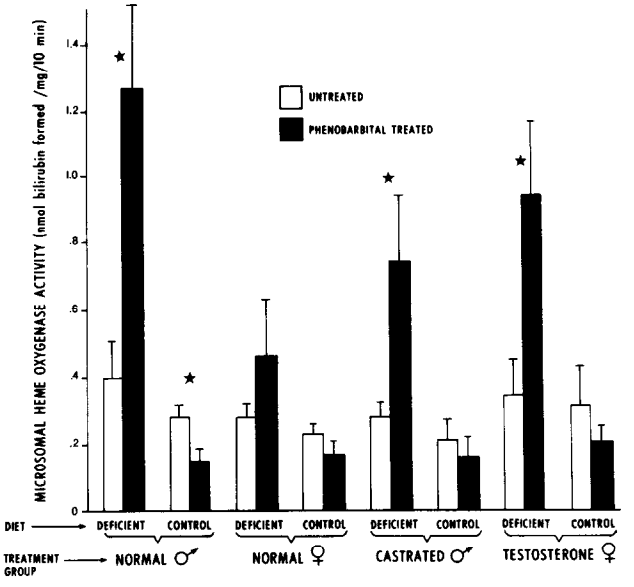

Fig. 3. Effects of sex, castration of male rats, and testosterone treatment of female rats on the phenobarbital-mediated stimulation of hepatic microsomal heme oxygenase activity in selenium deficiency. Rats treated with phenobarbital (80 mg/kg, i.p.) were studied 6 hr after injection. Values are means of four to six animals except for the phenobarbital-treated control male and castrated male rats which represent three animals each. Half brackets represent 1 S.D. Stars indicate significant differences ($P < 0.05$).

the untreated females. The rise was intermediate in the castrated males and testosterone-treated females.

Several other enzymes, known to be influenced by selenium status, were measured in the livers of the rats which did not receive phenobarbital (Table 1). Microsomal cytochrome P-450 content was unaffected by selenium status in these rats. The selenoenzyme glutathione peroxidase was virtually undetectable in the livers of all selenium-deficient rats. Its activity was twice as high in normal females as in normal males. Testosterone treatment of female rats lowered the glutathione peroxidase activity to levels observed in unoperated males, whereas castration of male rats had no effect on it. Glutathione *S*-transferase activities, which were measured with 1-CDNB and 1,2-DCNB, were unaffected by castration of male rats or testosterone treatment of female rats fed a control diet. Selenium deficiency caused a rise in glutathione *S*-transferase activity in unoperated males but not in other groups.

DISCUSSION

These results indicate that selenium-deficient female rats, selenium-deficient castrated male rats and selenium-deficient testosterone-treated female rats all have a defect in hepatic heme metabolism similar to the one described in selenium-deficient male rats [3]. Based on the relative increase in hepatic microsomal heme oxygenase activity due to phenobarbital treatment, this defect appears to be much less severe in selenium-deficient females than in selenium-deficient males. Castration of selenium-deficient males seems to lessen the defect. Testosterone-treatment of selenium-deficient females seems to worsen it. The cause of this sex-dependent effect is unknown, although there are several plausible explanations. It could be due to a greater heme turnover rate in male rat liver than in female rat liver, although we know of no data suggesting such increased turnover in males. A quantitative difference in response to phenobarbital is another possible explanation for our observations.

It seems likely that the sex-dependent effect is more related to selenium requirement and metabolism than to heme metabolism. All the animals in the present study that were fed the selenium-deficient diet had very low but comparable glutathione peroxidase activities (Table 1). However, our previous study suggested that the effect of selenium deficiency on heme metabolism is not mediated by glutathione peroxidase but by an as yet uncharacterized factor with a rapid turnover [3]. Thus, the known higher selenium requirement of male rats than of females could have resulted in a more severe selenium deficiency in males, with a consequently greater effect on the factor affecting heme metabolism.

Activity of glutathione peroxidase in the rat liver 105,000 *g* supernatant fraction is twice as high in females as in males [14] (Table 1). The reason for this is unknown, but it seems to be related to the presence of female hormones since castrated males, and females whose endogenous sex hormone production has been suppressed by testosterone treatment, have levels of glutathione peroxidase similar to those in unoperated males.

When we first made the observation that glutathione *S*-transferase activity increases in male rat liver in selenium deficiency, we postulated that the increase compensated for the fall in the selenium-

Table 1. Effect of selenium deficiency on some hepatic enzyme activities in male, female, castrated male, and testosterone-treated female rats*

	Microsomal cytochrome P-450 (nmoles/mg)	Glutathione peroxidase (μmoles NADPH oxidized/mg/min)	Glutathione S-transferase (μmoles conjugated/mg/min)		Weight† (g)
			1-CDNB	1,2-DCNB	
Male					
Deficient	0.93 ± 0.25	0.003 ± 0.001‡	1.00 ± 0.23§	0.07 ± 0.01‖	223 ± 35
Control	0.86 ± 0.10	0.28 ± 0.03‡	0.52 ± 0.07§	0.04 ± 0.01‖	222 ± 23
Female					
Deficient	0.62 ± 0.04	0.005 ± 0.001¶	0.68 ± 0.15	0.04 ± 0.01	176 ± 25
Control	0.65 ± 0.10	0.63 ± 0.03¶	0.56 ± 0.04	0.03 ± 0.01	181 ± 26
Castrated male					
Deficient	0.72 ± 0.19	0.004 ± 0.002**	0.68 ± 0.16	0.06 ± 0.02	174 ± 34
Control	0.64 ± 0.05	0.28 ± 0.02**	0.63 ± 0.06	0.05 ± 0.01	203 ± 20
Testosterone female					
Deficient	0.52 ± 0.08	0.005 ± 0.001††	0.66 ± 0.06	0.04 ± 0.01	177 ± 27
Control	0.60 ± 0.15	0.34 ± 0.03††	0.59 ± 0.15	0.04 ± 0.01	178 ± 16

* Values are means ±S.D. Cytochrome P-450 and enzyme activities were measured in livers of rats not receiving phenobarbital (n = four to six per group) whose microsomal heme oxygenase values are shown in Fig. 3.
† Weights of all rats (n = seven to ten per group) whether given phenobarbital 6 hr before being killed or not.
‡, §, ‖, ¶, **, †† Values with the same superscript are significantly different, $P < 0.05$.

dependent glutathione peroxidase activity [5]. However, in spite of dramatic decreases in glutathione peroxidase activity in livers of females, castrated males, and testosterone-treated females due to selenium deficiency, no increase in glutathione *S*-transferase activity occurred (Table 1). This casts doubt on our hypothesis that the increased activity observed in males is related to the fall in selenium-dependent glutathione peroxidase activity.

The results presented here demonstrate the influence of sex on the effect of selenium deficiency on a number of rat hepatic enzyme activities. Most of the effects of selenium deficiency on these activities are more prominent in male rats than in female, castrated male, or testosterone-treated female rats. This suggests that the male rat is likely to be the most sensitive model for the study of biochemical changes in selenium deficiency.

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REFERENCES

1. D. G. Hafeman, R. A. Sunde and W. G. Hoekstra, *J. Nutr.* **104**, 580 (1974).
2. R. F. Burk and B. S. S. Masters, *Archs Biochem. Biophys.* **170**, 124 (1975).
3. M. A. Correia and R. F. Burk, *J. biol. Chem.* **253**, 6203 (1978).
4. R. Tenhunen, H. S. Marver and R. Schmid, *J. biol. Chem.* **244**, 6388 (1969).
5. R. A. Lawrence, L. K. Parkhill and R. F. Burk, *J. Nutr.* **108**, 981 (1978).
6. W. B. Jakoby, in *Advances in Enzymology* (Ed. A. Meister), Vol. 46, p. 383. John Wiley, New York (1978).
7. I. Listowsky, K. Kamisaka, K. Ishitani and I. M. Arias, in *Glutathione: Metabolism and Function* (Eds. I. M. Arias and W. B. Jakoby), p. 233. Raven Press, New York (1976).
8. G. Siami, A. R. Schulert and R. A. Neal, *J. Nutr.* **102**, 857 (1972).
9. R. A. Lawrence and R. F. Burk, *J. Nutr.* **108**, 211 (1978).
10. P. Raj and R. W. Estabrook, *Pharmacologist* **12**, 261 (1970).
11. R. A. Lawrence and R. F. Burk, *Biochem. biophys. Res. Commun.* **71**, 952 (1976).
12. W. H. Habig, M. J. Pabst and W. B. Jakoby, *J. biol. Chem.* **249**, 7130 (1974).
13. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
14. R. E. Pinto and W. Bartley, *Biochem. J.* **112**, 109 (1969).