Biochemical Pharmacology, Vol. 17, pp. 327–330. Pergamon Press. 1968. Printed in Great Britain

Sex difference in the stimulatory effect of drugs on carbohydrate metabolism through the glucuronic acid pathways studied on the rat

(*Received* 21 *August* 1967; *accepted* 11 *October* 1967)

THE STIMULATORY effect of drugs on the biosynthesis of L-ascorbic acid, D-glucaric acid and L-xylulose[1–4], can be explained by an increased formation of the common intermediate D-glucuronic acid or its lactone from UDPG.[5] This effect results in an increased urinary excretion of the mentioned products by treated mammals. In studies of this phenomenon we observed that male rats respond more to drug treatment than female rats. Barbital, chlorophenothane, nikethamide and chloretone were studied. These drugs displayed a strong stimulatory effect on L-ascorbic acid excretion. Urinary excretion of D-glucaric acid was studied only in the case of barbital.

Adult rats were treated orally on three successive days, with 50, 100, and 150 mg/kg sodium barbital respectively. Before treatment no significant difference was observed between males and females in the L-ascorbic acid and D-glucaric acid excretion. On the days of drug treatment there was an increase in L-ascorbic acid excretion, which was significantly higher in males than in females. D-glucaric acid excretion, measured only on the third day of treatment was found to be increased. Again the increase was higher in males than in females (Fig. 1). Chlorophenothane, nikethamide and chloretone gave as far as the urinary excretion of L-ascorbic acid is concerned, the same type of sex difference. The data are summarized in Table 1.

TABLE 1. SEX DIFFERENCE IN L-ASCORBIC ACID RESPONSE OF RATS TO DRUG TREATMENT

Treatment	Excretion of L-ascorbic acid, μmole/24 hr/100 g rat*					
	before treatment			after treatment		
	male	female	P value†	male	female	P value†
Nikethamide 75 mg/kg orally at zero time and at 8 hr	1·9 ± 0·8 (6)	1·3 ± 0·6 (8)	N.S.	13·9 ± 5·9	8·1 ± 2·8	S.
Chlorophenothane 30 mg/kg orally at zero time	1·4 ± 0·8 (7)	1·4 ± 0·6 (8)	N.S.	4·9 ± 2·1	2·2 ± 0·6	S.
Chloretone 150 mg/kg orally at zero time and at 24 hr	2·3 ± 0·7 (5)	1·4 ± 0·9 (7)	S.	33·7 ± 5·9	20·4 ± 3·6	S.
				Increase‡:		
				31·4 ± 5·8	19·1 ± 3·6	S.

* Collection of urine was started immediately after first dose; after chloretone treatment urine was collected after last dose. Means with S.D. are shown; numbers in parentheses indicates number of animals on which each mean is based.

† P values were obtained by applying the two-sided Wilcoxon two-sample test: P value $\leqslant 0{\cdot}05$: S.; P value $> 0{\cdot}05$: N.S.

‡ Since in this case the nontreated male and female rats showed a significant difference also, the increase in L-ascorbic acid excretion (value after treatment minus value before treatment) was tested.

MATERIALS AND METHODS

Young adult male and female Wistar rats of the same age were used. Males and females weighed respectively 280–320 and 190–210 g; castrated males and females weighed 200–230 g. The animals were maintained on L-ascorbic acid free Hope Farms Laboratory Diet for rats with free access to water.

Urine was stored below 0°. For determination of L-ascorbic acid, urine was collected in 10% oxalic acid solutions. L-ascorbic acid was determined by the 2,6-dichlorophenolindophenol method, and D-glucaric acid by its specific inhibitory effect after acid treatment upon β-glucoronidase.[10]

In the literature, data on sex differences concerning drug metabolism are given.[6] As a rule males show a higher activity in this respect than females. Castration of both sexes results in an elimination of this difference, while the difference could be restored by hormone treatment. Therefore, it was of interest to determine whether there is a dependency on sex hormones in the stimulatory effect on carbohydrate metabolism. Immature male and female rats at the age of about 7 weeks were castrated.

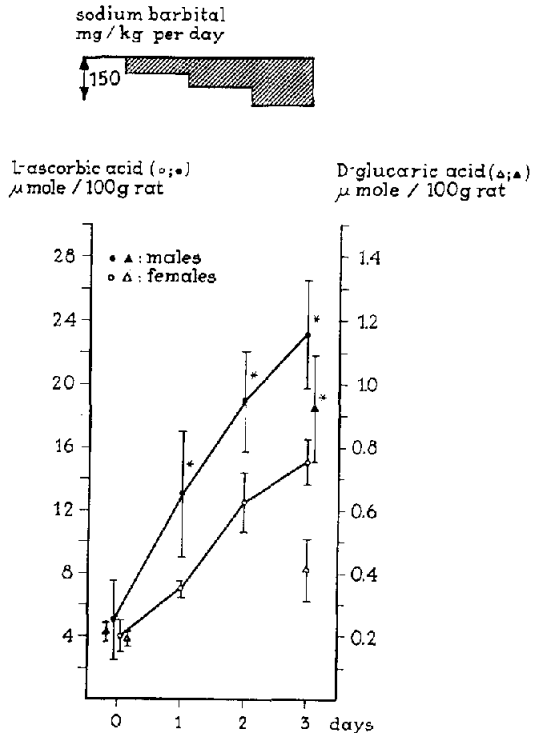

FIG. 1. Effect of barbital on the urinary excretion of L-ascorbic acid and D-glucaric acid by male and female rats. Rats were treated with sodium barbital as shown in figure. Each symbol corresponds with 24-hr urine collected after drug administration. Each value represents mean ± S.D. for 8 rats. Asterisk denotes a significant difference between males and females (P value $< 0{\cdot}05$; two-sided Wilcoxon two-sample test). Both the increase in L-ascorbic acid excretion and the increase in D-glucaric acid excretion after treatment with sodium barbital are significantly greater in males than in females.

After they were grown to adult animals no difference in L-ascorbic acid excretion was observed. Treatment with barbital resulted in an increase in L-ascorbic acid excretion; however, no significant difference was observed in both "sexes" (Fig. 2, first section). After barbital treatment was stopped, the "males" were treated continuously until the end of the experiment with estradiol and the "females" with testosterone. About 24 days after starting hormone treatment a little but significantly higher L-ascorbic acid excretion was observed from the estradiol-treated castrated "males". After administration of barbital to these animals the L-ascorbic acid excretion by testosterone-treated, castrated "females" increased more than by estradiol-treated, castrated "males" (Fig. 2, second section). After the last dose of barbital a significantly lower excretion was observed in estradiol-treated, castrated "males". As far as D-glucaric acid excretion is concerned no significant difference was observed between hormone treated "males" and "females" before barbital treatment. However, examination of the D-glucaric acid excretion on the third day of barbital treatment showed that the

excretion by estradiol-treated, castrated "males" was significantly lower. In summary, castration of male rats, followed by estradiol treatment and castration of female rats followed by testosterone treatment resulted in an inversion of the sex difference as far as L-ascorbic acid and D-glucaric acid excretion after barbital treatment is concerned. Compare Fig. 1 and Fig. 2.

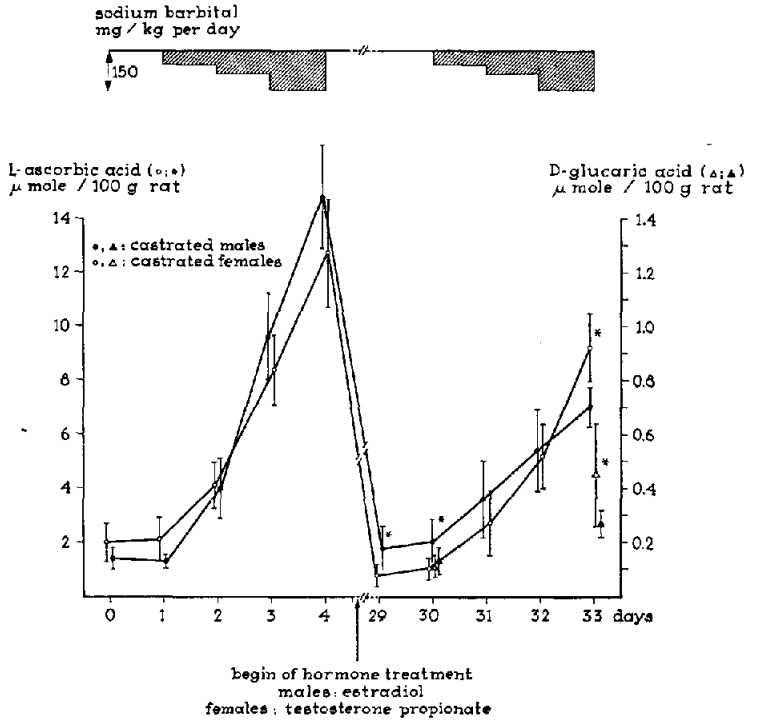

FIG. 2. Effect of barbital on the urinary excretion of L-ascorbic acid and D-glucaric acid by castrated male and female rats before and after treatment with sex hormones. Hormone treatment was started as indicated by arrow and continued during the whole experiment. To the castrated males, estradiol and to the castrated females testosterone propionate was given intramuscularly every other day in doses of 2 μg in 0·1 ml sesame oil. Further conditions are similar to those described in Fig. 1. After castration, no significant sex difference in the barbital-caused increase in L-ascorbic acid excretion is observed (see first section of the curves and compare with Fig. 1). In the hormone-treated animals the values after administration of 150 mg/kg sodium barbital again differ significantly, but now the testosterone-treated females show the higher L-ascorbic acid and the higher D-glucaric acid excretion (see second section of the curves and compare with Fig. 1).

It was observed by Nitze and Remmer[7] that the rat after treatment with barbital, does not show sex difference in the urinary excretion of D-glucuronic acid. We could confirm this observation. The absence of sex difference in the urinary excretion of D-glucuronic acid after barbital treatment may indicate that a sex difference in the formation of intracellular D-glucuronic acid in rat liver does not occur. The terminal step in the D-glucaric acid formation in liver is catalysed by D-glucuronolactone dehydrogenase.[8] It has been shown in rat and mice that liver D-glucuronolactone dehydrogenase activity is higher in males than in females.[9] The difference appears at the time of sexual maturity. If the higher enzyme activity in male is correlated with a higher capacity *in vivo* to form D-glucaric acid from D-glucuronolactone the sex difference in the excretion of D-glucaric acid after stimulation can be explained as follows: During stimulation by drug an enhanced formation of D-glucuronolactone occurs in male and female rat liver possibly to the same extent. This D-glucuronolactone is partly metabolized via the D-glucaric acid pathway. The higher capacity in male rat liver to form D-glucaric

acid from D-glucuronolactone results in a higher D-glucaric acid production, leading to a higher D-glucaric acid excretion. A similar explanation might fit for the sex difference in the L-ascorbic acid response upon stimulation. Recently sex difference in the activity of liver glucuronolactone reductase and of liver L-gulonolactone oxidase has been shown by Stubbs and McKernan.[11]

Acknowledgements.—The technical assistance of Miss C. G. M. van der Knaap and Miss G. H. M. van Kuppevelt is acknowledged.

Department of Pharmacology,
University of Nijmegen,
Nijmegen,
The Netherlands

E. M. AARTS

REFERENCES

1. J. J. BURNS, A. H. CONNEY, C. EVANS, G. R. MARTIN and D. TALLER, *J. Pharmac. exp. Ther.* **129**, 132 (1960).
2. M. ENKLEWITZ and M. LASKER, *J. biol. Chem.* **110**, 443 (1935) .
3. C. A. MARSH and L. M. REID, *Biochem. biophys. Acta* **78**, 723 (1963).
4. E. M. AARTS, *Biochem. Pharmac.* **14**, 359 (1965).
5. C. EVANS, A. H. CONNEY, N. TROUSOF and J. J. BURNS, *Biochim. biophys. Acta* **41**, 9 (1960).
3. B. B. BRODIE, *J. Pharm. Pharmac.* **8**, 1 (1956).
7. H. R. NITZE and H. REMMER, *Arch. exp. Path. Pharmak.* **242**, 555 (1963).
8. C. A. MARSH, *Biochem. J.* **87**, 82 (1963).
3. C. A. MARSH and A. J. CARR, *Clin. Sci.* **28**, 209 (1965).
10. C. A. MARSH, *Biochem. J.* **83**, 77 (1963).
11. D. W. STUBBS and J. B. MCKERNAN, *Proc. Soc. exp. Biol Med.* **125**, 1326 (1967).

Biochemical Pharmacology, Vol. 17, pp. 330–332. Pergamon Press. 1968. Printed in Great Britain

Effect of sodium diethylbarbiturate on xanthine dehydrogenase

(*Received* 4 *July* 1967; *accepted* 14 *September* 1967)

IN A PREVIOUS paper it was shown that xanthine oxidase and dehydrogenase activities (XO, XD) decrease sharply in the blood serum and brain of rats 3 hr after the beginning of narcosis produced by sodium diethylbarbiturate (Medinal).[1] Since XO is associated with lipids through lipoprotein bonds[2,3] and that there is a correlation between enzymatic activity and unsaturated fatty acids[4] it seemed of interest to know if the breakage of the lipid-enzyme association *in vitro* and *in vivo* could account for some of the inhibition produced by barbiturates. In the present note results obtained with the purified enzyme submitted to different treatments are reported. Carbon tetrachloride exerts *in vivo* a dissociation of the lipid enzyme complex increasing its activity. Therefore, the experiments with CCl_4 were also included to show the effect on the barbiturate *in vivo*.

Medinal is inhibitory *in vitro* when incubated with serum or brain homogenates for 30–60 min at 37° but only slightly if not incubated. However, when liver preparations were submitted to treatments which liberate the lipid material before Medinal addition such as heating at 56°/30 min, treatment with *Tween 80* (1%)/30 min, extraction with butanol, addition of sodium desoxycholate (1·5 mg/ml