Biochemical Pharmacology, 1962, Vol. 11, pp. 221–227 Pergamon Press Ltd., Printed in Great Britain.

INFLUENCE OF SEX DIFFERENCE ON THE PHARMACOLOGICAL ACTION AND METABOLISM OF SOME DRUGS*

R. KATO, E. CHIESARA and G. FRONTINO

Institute of Pharmacology, University of Milan, Milan, Italy

(*Received* 2 *October* 1961; *accepted* 14 *November* 1961)

Abstract—A sex difference in the pharmacological effects of pentobarbital, carisoprodol, strychnine, OMPA in adult rats has been demonstrated. These sex differences almost disappeared after the administration of SKF 525 A. The differences were also demonstrated *in vitro* using liver slices and microsomal fractions, and by *in vivo* metabolic studied. They were modified by castration and by sex hormone treatments. The anabolic action of male sex hormones appears to account for the increased enzyme activities in male rats.

IN RECENT years, it has become generally recognized that the breakdown of drugs by the liver is of great importance in determining the duration of drug action.[1] Liver damage or administration of SKF 525 A markedly prolongs the duration of drug action by impairment of drug metabolism.[2] A sex difference in the pharmacological effects of some drugs (e.g. strychnine and some barbiturates) is already well known. Many studies have been done on this sex difference in the action of some barbiturates and it is attributed to the high activity of enzymes concerned in barbiturate metabolism in the male adult rats.[2–7] The sex difference in strychnine toxicity was studied by Poe *et al.* (1937), but it has been considered to be due to a difference in the susceptibility of the central nervous system in male and female adult rats.[8, 9] Indeed the time of onset of the strychnine convulsion (7–8 min after intraperitoneal injection) was probably too short for the marked difference in toxicity to be explained by a metabolic factor. We have recently demonstrated that the actions of some drugs (called the induced drugs; hexobarbital, pentobarbital, meprobamate, carisoprodol, strychnine, picrotoxin and octamethylpyrophosphoramide) are modified by pretreatment with other drugs (e.g., phenobarbital, thiopental, phenaglycodol, glutethimide, nikethamide and meprobamate; the inducing drugs).[11, 13, 14] The mechanism by which this modification was brought about, might be an increased metabolism of the drugs.[10–15] Those observations suggest that the metabolic factor is most important in determination of the duration and the intensity of the drug, especially, concerned in the inducing drug. It was therefore supposed that the sex difference in pharmacological effects of some induced drugs may be due to the different metabolic activities in female and male rats. The present work is an attempt to elucidate this possibility.

* Presented at the First International Pharmacological Meeting, Stockholm, 1961.

MATERIALS AND METHODS

Both sexes of Sprague–Dawley rats were used, sodium pentobarbital, sodium hexobarbital, strychnine sulphate, picrotoxin, octamethylpyrophosphoramide (OMPA) and SKF 525 A (β-diethylaminoethyldiphenylpropylacetate hydrochloride)* were given intraperitoneally in doses of 1 ml/kg of distilled water. Carisprodol were given intraperitoneally in a suspension of 1 per cent methylcarboxycellulose (1 ml/kg). Estradiol, testosterone and 4-chlortestosterone were injected intraperitoneally in 1 ml/kg of arachid oil. Ninety-minutes sleeping-time dose of pentobarbital, 90 min paralysis dose of carisoprodol, and 50 per cent lethal dose of strychnine and OMPA were determined according to the methods of Lichtfield and Wilcoxon.[18]

Determination of *in vivo* metabolism of carisoprodol was carried out as previously reported.[19] *In vitro* metabolism of the drugs was determined as previously reported.[20, 21] Sleeping time after pentobarbital and paralysis after carisoprodol were taken as the duration of loss of the righting reflex.

RESULTS

The sex difference in the pharmacological effects of pentobarbital, carisoprodol strychnine and OMPA in female and male rats is summarized in Table 1.

TABLE 1. SEX DIFFERENCE IN THE PHARMACOLOGICAL EFFECTS OF SOME DRUGS AND EFFECT OF SKF 525 A ON THE SEX DIFFERENCE

Sex	Pentobarbital sleeping-time for 90 min (mg/kg)	Carisoprodol paralysis for 90 min (mg/kg)	Strychnine LD_{50} mg/kg)	OMPA LD_{50} (mg/kg)
Female	25·8	193	1·61	24
Male	34·2	241	2·61	5·5
Female + SKF 525 A	19·6	132	1·40	62
Male + SKF 525 A	20·5	143	1·37	58

Experiments were on female and male rats for the Sprague–Dawley strain weighing about 200 g and 280 g, respectively. SKF 525 A (50 mg/kg, 5 mg/kg for OMPA) was injected 30 min before the injection of pentobarbital, carisoprodol and OMPA and 50 min before the strychnine injection. The values given represent those obtained from at least twenty-six animals.

The effect of pentobarbital is shown as the dose of the drug which produced the sleeping time for 90 min after intraperitoneal injection (SD 90 min). The SD 90 min of pentobarbital in the female rats is 25·8 mg/kg, while in the male rats it is 34·2 mg/kg. To express the difference in another way, the sleeping time after pentobarbital injection (28 mg/kg, i.p.) is 113 min in the female rats and 48 min in the male rats.

The effect of carisoprodol is also shown as the dose of the drug which produced paralysis for 90 min (PD 90 min). The PD 90 min of carisoprodol in the female rats is 193 mg/kg while in the male rats it is 241 mg/kg. The duration of paralysis after carisprodol injection (200 mg/kg, i.p.) is 108 min, in the female, and 43 min in the male rats.

The effects of strychnine and OMPA are shown as the 50 per cent lethal dose (LD_{50}). The LD_{50} of strychnine in the female rats is 1·61 mg/kg, while in the male

* SKF525A was kindly supplied by Dr. H. E. Duell (Smith, Klein & French Laboratories, Philadelphia.)

rats it is 2·61 mg/kg. The mortality after 2·0 mg/kg strychnine (i.p.) is 84 per cent in the female rats and 8 per cent in the male rats.

The LD_{50} of OMPA in the female rats is 24 mg/kg, while in the male rats it is 5·5 mg/kg. The mortality after 12 mg/kg OMPA (i.p.) is 2 per cent in the female rats and 96 per cent in the male rats.

The increased effects of pentobarbital, carisoprodol, strychnine and picrotoxin and the decreased effects of OMPA after SKF 525 A have been confirmed in our laboratory.[11]

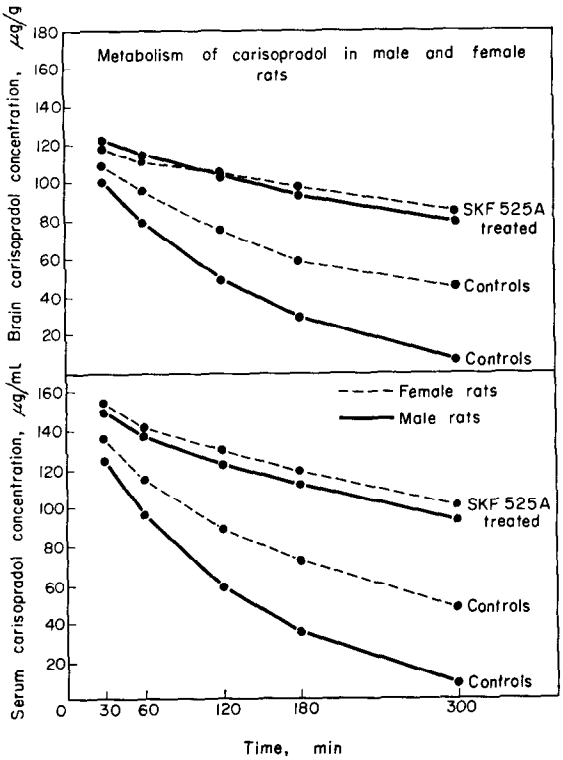

FIG. 1. Metabolism of carisoprodol in male and female rats. Experiments were on female and male rats of the Sprague–Dawley strain weighing about 200 g and 280 g, respectively. 150 mg/kg of carisoprodol were injected intraperitoneally 30 min after the injection of SKF 525 A (50 mg/kg, i.p.).

Experiments were therefore carried out using SKF 525 A to examine the possibility that those different pharmacological effects are due to the different metabolic activities of female and male rats.

As is shown in Table 1, the sex difference in the sleeping time after pentobarbital, in the paralysis after carisoprodol and in the strychnine and OMPA toxicities disappeared after SKF 525 A administration (50–5 mg/kg, i.p. 30 min or 50 min before). Similar results were obtained with picrotoxin toxicity and hexobarbital sleeping time.

Those results suggest that the sex difference in the pharmacological effects of pentobarbital, carisoprodol, strychnine, and OMPA may be due to different metabolic activity in female and male rats.

The *in vivo* metabolism of the drugs in female and male rats was determined after the injection of carisoprodol.

The decrease of carisoprodol concentrations in serum and brain in the male rats was much faster than in the female rats, but this difference disappeared after pretreatment with SKF 525 A (50 mg/kg, i.p., 30 min before) (Fig. 1).

This result suggests that the sex difference in the carisoprodol paralysis is due to the rapid metabolism in liver of the male rats but it is not due to a rapid elimination of the drug.

Table 2 also shows the relationship between the duration of paralysis and carisoprodol concentrations in serum and brain at the end of paralysis.

TABLE 2. CARISOPRODOL CONCENTRATIONS IN SERUM AND BRAIN OF MALE AND FEMALE RATS AT THE END OF PARALYSIS

Sex	Duration of paralysis (min)	Carisoprodol concentration	
		Serum (μg/ml)	Brain (μg/g)
Male	43 $\pm$ 3·4	118 $\pm$ 3·7	105 $\pm$ 3·5
Female	108 $\pm$ 7·3	131 $\pm$ 4·1	117 $\pm$ 3·2

200 mg/kg carisoprodol was injected intraperitoneally. The rats were killed at the end of paralysis and the carisoprodol concentrations in serum and brain determined. The values given represent averages obtained from ten animals.

These concentrations are the same, suggesting that there is no difference in sensibility to carisoprodol between female and male rats.

The sex difference in the pharmacological effects of pentobarbital, carisoprodol, strychnine and OMPA could only be demonstrated in adult rats. No such difference could be demonstrated in immature rats or in adult mice or guinea-pigs.

The results obtained in *in vitro* experiments also confirmed the results obtained in the pharmacological experiments. A typical example is shown in Fig. 2 where it is seen that the metabolic difference due to sex may begin about 40–50 days after birth.

These results were obtained with liver slices and similar results were obtained with the liver microsomal fraction.

The fact that the sex difference only exists in adult rats suggests that it may be due to an anabolic action of male sex hormones. This possibility was examined by modifying the sex hormone condition of female and male rats.

Fig. 3 shows that castration decreases the high activity of the liver enzyme responsible for breakdown of carisoprodol in male rats, while spaying did not modify the enzyme activity in female rats. Treatment with testosterone increased the enzyme activity in both male and female castrated rats. Treatment with 4-chlortestosterone, which has a potent anabolic action with a very weak androgenic action, also increased the enzyme activity. On the other hand, treatment with estradiol did not modify the enzyme

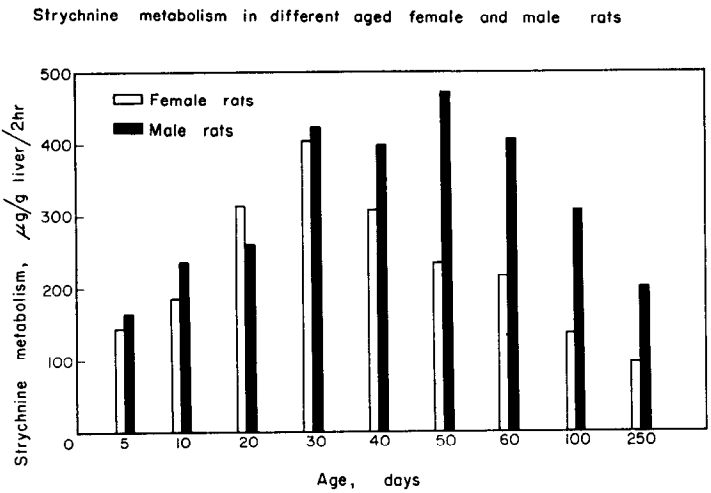

FIG. 2. Strychnine metabolism in female and male rats at different ages. Experiments were on the liver slices obtained from female and male rats of the Sprague–Dawley strain. Enzyme activity was represented by μg of the substance metabolized in 1 g of the liver slices during an incubation of 2 hr.

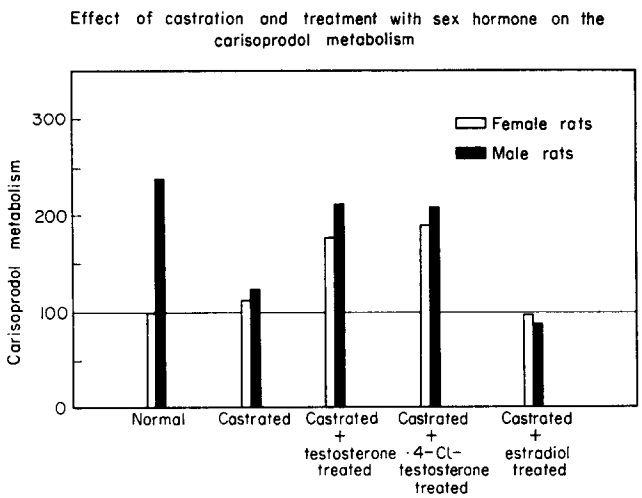

FIG. 3. Effect of castration and treatment with sex hormones on carisoprodol metabolism. Experiments were on female and male rats of the Sprague–Dawley strain weighing about 200 g and 280 g, respectively. The rats were castrated 25 days before and treated with 200 μg/kg of estradiol or 2·5 mg/kg of testosterone or 4-chlortestosterone for 15 days before the sacrifice. The enzyme activities were determined in the microsomal fraction. The values given represent averages obtained from at least six animals and expressed per cent of normal female rats (56 μg/g liver per 2 hr).

activity in castrated male and female rats. The similar results were obtained with *in vivo* experiments on the paralysis after carisoprodol (Fig. 4).

With strychnine, results similar to those obtained with carisoprodol were observed.

In some experiments, the supernatant obtained from the male rats was added to the microsomes obtained from female rats, or vice versa. The results did not suggest the presence of an activator or inhibitor which might have accounted for the sex difference.

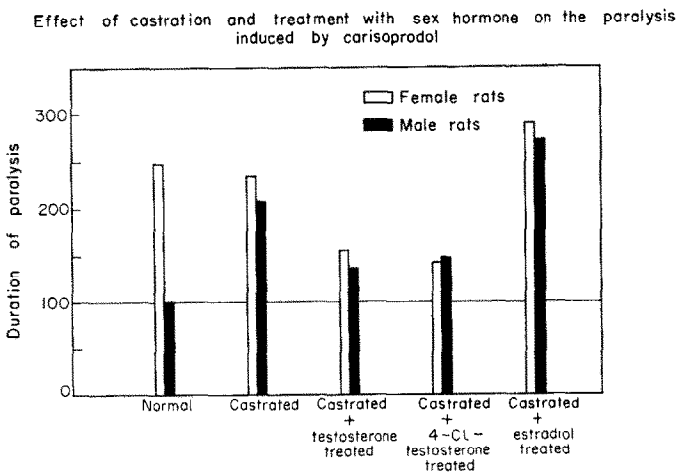

FIG. 4. Effect of castration and of treatment with sex hormones on the paralysis induced by carisoprodol. The experimental conditions are same as in Fig. 3. The rats were injected intraperitoneally with 200 mg/kg of carisoprodol. The values given represent averages obtained from at least twelve animals and expressed as per cent of normal female rats (109 min).

DISCUSSION

The results of this study show that there is sex difference in adult female and male rats concerned with the pharmacological effects of pentobarbital, carisporodol, strychnine, picrotoxin and OMPA and those differences only observed adult rats.

The high activity of the drug-metabolizing enzymes in adult rats may be due to an anabolic action of testosterone, because such as 4-chlortestosterone, which has a potent anabolic action with a very weak androgenic action, also shows an increase of the enzyme activity. The opposite result found in the toxicity of strychnine and OMPA is due to the fact that OMPA itself has no effect on cholinesterase activity except after being decomposed by the liver.[25] The exchange of the microsomes and the supernatant indicate that the factors responsible for high activities in male rats present only in the microsomes. On the other hand, there are the following analogies among the metabolism of pentobarbital, hexobarbital, carisoprodol, strychnine, picrotoxin, and OMPA.

(1) The enzymes responsible for their metabolism was only found in the microsomes of liver.[22–26]

(2) The enzymes require TPNH and O_2.[22–26]

(3) The presence of a sex difference in adult rats concerning the enzyme activities.

(4) The enzyme activities are increased by pretreatment with phenobarbital, phenaglycoeol, or glutethimide.[10, 12, 15–17, 26]

(5) The enzymes activities are inhibited by SKF 525 A, marsilid, or isoniazid.[2, 11, 22–25, 27]

Recently, it was reported that the administration of phenobarbital increases the protein content of whole liver and of microsomes.[17]

Those results suggest that there are some similarities between the mechanism by which produces an increase of activities of the microsomal drug-metabolizing enzymes by the inducing drugs and the anabolic hormones.

However, the inducing drugs take 12–18 hr to produce their effect while the anabolic hormones take at least 4–5 days, and they can produce an increase of the enzyme activities only in rats, while the inducing drugs do so in mice and in guinea-pigs also.

REFERENCES

1. B. B. Brodie, J. R. Gillette and B. N. La Du, *Ann. Rev. Biochem.* **27**, 427 (1958).
2. B. B. Brodie, *J. Pharm., Lond.* **8**, 1 (1956).
3. H. G. O. Holock, M. A. Kanan, L. M. Mills and E. L. Smith, *J. Pharmacol.* **60**, 323 (1937).
4. W. M. Moir, *J. Pharmacol.* **59**, 68 (1937).
5. M. Crevier, A. D'Iorio and E. Robillard, *Rev. Canad. Biol.* **9**, 336 (1950).
6. J. Pellerin, A. D'Iorio and E. Robillard, *Rev. Canad. Biol.* **13**, 257 (1954).
7. R. A. Edgren, *Experientia* **13**, 86 (1957).
8. C. F. Poe, J. F. Suchy and N. F. Witt, *J. Pharmacol.* **58**, 239 (1937).
9. C. F. Poe and J. F. Suchy, *Arch. Int. Pharmacodyn.* **86**, 449 (1951).
10. R. Kato, *Atti Soc. lombardi. Sci. Med. Biol.* **14**, 777 (1959).
11. R. Kato, *Atti Soc. lombardi. Sci. Med. Biol.* **14**, 783 (1959).
12. R. Kato, *Med. Exp.* **3**, 95 (1960).
13. R. Kato, *Proceeding of 1st Symp. Europ. Med. Eznymol.* 1960, p. 364. Karger, Basel.
14. R. Kato, *Arzneimittel Forsch.* **11**, 797 (1961).
15. R. Kato, E. Chiesara, *Brit. J. Pharmacol.* In press.
16. H. Remmer, *Arch. exp. Path. Pharmak.* **237**, 296 (1959).
17. A. H. Conney, C. Davison, R. Gastel, J. J. Burns, *J. Pharmacol.* **131**, 1 (1960).
18. T. T. Lichtfield and F. Wilcoxon, *J. Pharmacol.* **99**, 49 (1949).
19. R. Kato, P. Vassanelli and G. Frontino, *Med. Exp.* In press.
20. R. Kato, E. Chiesara and P. Vassanelli, *Jap. J. Pharmacol.* In press.
21. R. Kato, E. Chiesara and P. Vassanelli, *Biochem. Pharmacol.* In press.
22. J. R. Copper and B. B. Brodie, *J. Pharmacol.* **114**, 409 (1955).
23. J. R. Copper and B. B. Brodie, *J. Pharmacol.* **120**, 75 (1957).
24. R. H. Adamson and J. R. Fouts, *J. Pharmacol.* **127**, 87 (1959).
25. A. N. Davison, *Nature, Lond.* **174**, 1056 (1954).
26. R. Kato, E. Chiesara and P. Vassanelli. In preparation.
27. R. Kato, E. Chiesara and P. Vassanelli. Unpublished data.