Influence of Estradiol on the Disposition of Chlorpromazine in the Rat

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The treatment of male rats with estradiol resulted in an increased hypothermic response to chlorpromazine. This increased pharmacologic response appears to be due to a decreased metabolism of chlorpromazine as indicated by the increased blood levels of S-35 after S-35 chlorpromazine administration and a decreased *in vitro* metabolism of chlorpromazine by liver microsomes from treated rats. No significant change was observed in the total liver or microsomal protein content. There is indication that decreased NADPH oxidase activity may be involved.

MOST OF the studies involving the effect of sex hormones on drug metabolism were attempts to explain sex differences in drug responses. Axelrod (1) studied the effect of sex hormones on the *in vitro* *N*-demethylation of methadone, meperidine, and morphine in liver microsomes. The treatment of male rats with estradiol decreased this metabolic activity. Murphy and DuBois (2) found that pretreatment with estrogen had a similar effect on the enzymes responsible for the oxidation of gluthion. Estrogen also decreases the metabolism of hexobarbital in liver microsomes from male rats (3, 4).

Hyvert *et al.* (5) reported that female patients responded more satisfactorily than male patients to chlorpromazine treatment. Also, male patients treated with estrogens had more satisfactory responses to chlorpromazine therapy than did males not on estrogen treatment. Berti and Cima (6) reported that lesser amounts of the demethylated chlorpromazine derivatives and/or their sulfoxides were detected in the livers and brains of female rats as compared to the same organs in male rats. Thus, the objective of this study was to determine the effect of estrogen treatment on hypothermia, tissue distribution, and metabolism of chlorpromazine in the rat.

METHODS

Male Holtzman rats, weighing 240 to 250 Gm., were given 25 mcg. of estradiol valerate in sesame oil by intramuscular injection at 5-day intervals and control animals received injections of sesame oil. The hypothermia was determined by measuring colonic temperature with a telethermometer and thermistor probe at 1-hr. intervals after an intraperitoneal injection of chlorpromazine hydrochloride.

The tissue uptake and plasma level studies were determined with S-35 chlorpromazine after 3 weeks of estradiol treatment. Five treated and five control animals were given intraperitoneal injections of chlorpromazine S-35 hydrochloride. The total dose of chlorpromazine, as well as the radioactivity, was determined by weight of the animal (10 mg./Kg. containing 8 μc.). One animal from each group was sacrificed by decapitation at 0.5, 1, 2, 4, and 8 hr. This procedure was repeated for 5 days using the same time schedule so that the animals at the appropriate intervals were sacrificed at the same time of day.

After centrifugation of the blood sample, 1 ml. of plasma was placed in a Wheaton vial and held for tissue digestion. Immediately after the collection of the blood, the brain was perfused by flushing 10 ml. of normal saline through the carotid arteries. A whole-brain homogenate was then prepared, and a 1-ml. aliquot, which was equivalent to 200 mg. of brain tissue, was transferred to a Wheaton vial. Liver samples were obtained from the median lobe.

The plasma and tissue samples were digested by a hyamine potassium hydroxide mixture. The samples were counted in a liquid scintillation spectrometer. The activity of the samples was corrected for counting efficiency with the use of C-14 as described by Emmerson *et al.* (7). The results of these studies were expressed as levels or uptake of S-35 phenothiazines since the radioactivity present was due not only to chlorpromazine but also to its metabolites.

An analysis of variance (ANOV) was used to analyze the data. The variances resulting from the interactions of treatments, days, and time intervals in the tissue uptake studies were all analyzed in the $5 \times 4 \times 2$ factorial analysis of variance design (5 time periods, 4 days, 2 treatments). The actual computation of the ANOV was accomplished by the IBM 7090 computer. For the computation of the F ratios a within-cell variance which consisted of all the sums of squares resulting from the day to day variation and any interactions involving days divided by their total degrees of freedom served as the error variance. For the purpose of testing the homogeneity variance between the treated and control groups at various time intervals an analysis of variance for simple effects was used (8).

The effect of estradiol on the food intake of the treated male rats was determined by conducting paired feeding tests. The *in vitro* metabolism of chlorpromazine in the $9000 \times g$ supernatant fraction of liver homogenates from nine male rats which had been treated with estradiol was compared with nine control rats. The 25-ml. reaction flasks which

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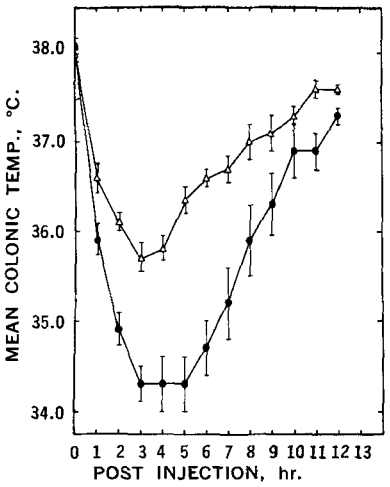

Fig. 1.—The effect of estradiol on chlorpromazine hypothermia (10 mg./Kg.). Mean of eight animals ± S.E.; 3 weeks of estrogen treatment schedule; 10 mg./Kg. chlorpromazine hydrochloride (by intraperitoneal injection). Key: △, control; ●, estradiol treated. Room temperature, 26–27°.

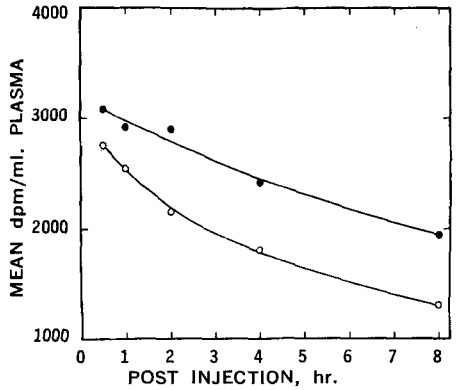

Fig. 2.—Plasma S-35 levels after chlorpromazine S-35 hydrochloride injection in estradiol-treated rats. Each point represents a mean of five rats. Key: O, control; ●, estradiol treated.

contained 1 ml. of the supernatant, 30 μm. of glucose-6-phosphate, 4 μm. of NADP, 50 μm. $MgCl_2$, 2 μm. of chlorpromazine HCl, and 50 μm. of nicotinamide were incubated in a Dubnoff metabolic incubator at 37° for 45 min. under an atmosphere of oxygen. The amount of chlorpromazine and chlorpromazine sulfoxide at the end of the incubation period was determined by the method of Salzman and Brodie (9) as modified by Gillette and Kamm (10).

The protein content of the liver and the lyophilized liver microsomes of treated and control rats was determined by employing the method of Lowry *et al.* (11). The NADPH oxidase activity of the lyophilized microsomes was determined by using the method of Gillette *et al.* (12).

RESULTS AND DISCUSSION

An increase in hypothermic response to chlorpromazine HCl (10 mg./Kg.) was evident in estradiol-treated male rats (Fig. 1). The treated rats in this study had been administered estradiol for 3 weeks. The mean colonic temperature for the treated rats at 3 hr. post injection was 34.2° as compared to the control mean of 35.6°.

At the onset of this study it was evident that the estradiol-treated rats did not gain weight as did their controls. Paired feeding tests revealed that the decreased growth rate was not due to a decreased food intake in the treated rats. At the end of 3 weeks of paired feeding tests, the treated group showed an increased hypothermic response to chlorpromazine while the paired-fed group did not. The appetite depressant action of estradiol, therefore, was not responsible for the hypothermic response.

The mean wet weight for the seminal vesicles of 10 rats treated with estradiol for 3 weeks was 35.5 ± 2.1 mg./100 Gm. body weight, while that of the controls was 155 ± 5.7 mg./100 Gm. body weight. The adrenal weights of the treated rats were increased with a mean of 23.1 ± 0.37 mg., while the mean weight of the controls was 14.9 ± 0.46 mg./100 Gm. body weight.

The implications of this adrenal hypertrophy in the metabolism of chlorpromazine were not evaluated in this study. Further studies along this line would be needed to determine whether there is any change in the blood levels of cortical steroids. Rupe *et al.*

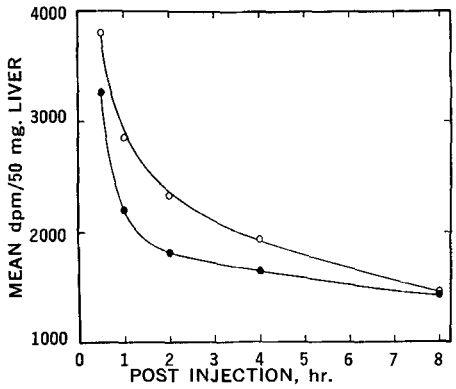

Fig. 3.—Liver S-35 levels after chlorpromazine S-35 hydrochloride injection in estradiol-treated rats. Each point represents a mean of four rats. Key: O, control; ●, estradiol treated.

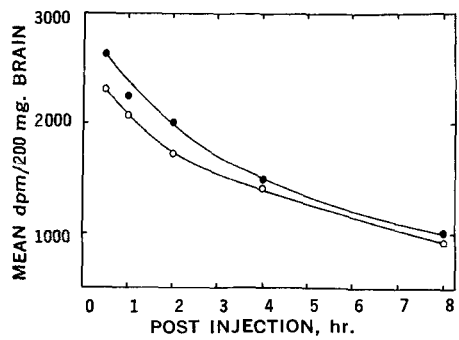

Fig. 4.—Brain S-35 levels after chlorpromazine S-35 hydrochloride injection in estradiol-treated rats. Each point represents a mean of four rats. Key: O, control; ●, estradiol treated.

TABLE I.—EFFECT OF ESTRADIOL ON THE *In Vitro* METABOLISM OF CHLORPROMAZINE IN MALE RATS

Treatment[a]	Liver Wt. / Body Wt. × 100	mcg. Protein/ mg. Liver (± S.E.)	Chlorpromazine Metabolized[b] (μm. ± S.E.)	Chlorpromazine Sulfoxide (μm. ± S.E.)
Estradiol (9)[c]	3.8 ± 0.10	212.8 ± 3.7	0.68 ± 0.04	0.13 ± 0.005
Control (9)	3.6 ± 0.03	207.5 ± 5.3	1.62 ± 0.08	0.23 ± 0.010

[a] Number of animals studied are in parentheses. [b] Per 500 mg. liver equivalent. [c] After 5 weeks of estradiol treatment (total of 200 mcg.).

TABLE II.—EFFECT OF ESTRADIOL TREATMENT ON LIVER AND MICROSOMAL PROTEIN CONTENT

Treatment[a]	Liver Wt. / Body Wt. × 100	mcg. Protein/ mg. Liver (± S.E.)	mcg. Microsomal Protein/Gm. Liver[b]	μm. NADPH Oxidized[c]/min./ mg. Protein
Estradiol (4)[d]	3.8 ± 0.10	217.7 ± 1.4	13.47	2.7
Control (4)	3.8 ± 0.03	225.0 ± 4.1	13.34	4.6

[a] Number of animals are in parentheses. [b] Average protein content for the pooled livers. [c] An average of four determinations. [d] After 7 weeks of estradiol treatment (a total of 275 mcg.).

(13) reported that administration of ACTH and corticosterone shortened the duration of action of hexobarbital by increasing the rate of drug metabolism.

The androgen antagonistic action of estradiol is probably involved in any effect estrogen may have on chlorpromazine metabolism. The administration of testosterone has been shown to increase the metabolism of various drugs in the female and immature male rats. Furthermore, results reported in the literature indicate that it is the anabolic activity of testosterone and not so much its androgenic activity which increases drug metabolism in rats (4, 14).

The mean plasma levels of S-35 phenothiazine in the treated rats were higher at all time intervals than the controls (Fig. 2). The *F* ratios for the variances between the treated and control groups and between the time intervals were significant at the 0.05 level. The analysis of variance for simple effects revealed that the variances between treated and control plasma levels at 2 hr. was significant at the 0.05 level.

The liver uptake of S-35 phenothiazines was decreased in the estradiol-treated rats as compared to the controls (Fig. 3). The variance between the treated and control groups was significant at the 0.05 level. The analysis of variance for simple effects revealed that the variance between treated and control groups at the 1-hr. time interval was significant at the 0.10 level.

The S-35 uptake in the brain homogenates of treated rats was slightly increased (Fig. 4), but was not statistically significant. However, significant qualitative changes in the metabolites may have taken place. Brain metabolites were not investigated.

The *in vitro* metabolism of chlorpromazine in the 9000 × *g* supernatant fraction of liver homogenates from the control male rats was more than twice that of the treated (Table I). The treated rats in this case had been administered estradiol for 5 weeks. The presence of an inhibitor in the homogenates from the treated rats was ruled out when the mixing of the two homogenates did not decrease the metabolic rate of the control microsomes beyond their microsomal potential.

The means of whole liver protein content of the treated and control rats were not significantly different at the 0.05 level using the Student *t* test (Table I). The liver to body weight ratios were not significantly different. Protein analysis of the pooled liver microsomes showed no difference between the treated and control rats (Table II).

These results indicate that the mechanism for the decreased chlorpromazine metabolism in the estradiol-treated rats is not a decreased enzyme synthesis of a magnitude which would be reflected in a change in total liver or microsomal protein.

The NADPH oxidase activity was decreased in the microsomes of the estradiol-treated rats (Table II) as compared to the controls. This decreased enzyme activity may account for the decreased chlorpromazine metabolism. Brodie *et al.* (15) and Gillette and Kamm (10) have suggested that the oxidation of NADPH may play an important role in drug metabolism. In view of a later report by Booth and Gillette (4), further studies are necessary to clarify the role of NADPH oxidase activity on drug metabolism.

SUMMARY

Chlorpromazine hypothermia is potentiated by estradiol treatment. The potentiation may be brought about by a decreased metabolism of the drug but is not reflected in the protein content of liver or liver microsomes.

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