Mechanism of Chlorpromazine-Induced Liver Nonprotein Sulfhydryl Depletion.* (31147)

J. E. WEAVER,† J. H. MENNEAR‡ AND T. S. MIYA
Purdue University, Department of Pharmacology, Lafayette, Indiana

Administration of several chemically unrelated materials, including chlorpromazine, or the exposure of animals to various stressful conditions has been shown to deplete liver nonprotein sulfhydryl (LNPSH)(1-7). These experiments were designed to study the mechanism by which chlorpromazine induces LNPSH depletion.

Glutathione is the primary alterable constituent of LNPSH(8). Therefore, changes in the concentration of the latter are generally considered to represent variations in glutathione concentrations. Measurements of adrenal ascorbic acid (AAA) levels were made concomitant with LNPSH determinations. Depletion of AAA was considered to be indicative of stress(9-11).

Female Holtzman rats (175-225 g) were employed throughout the project, and were housed in air-conditioned quarters. Food and water were supplied *ad libitum* until 4 hours before sacrifice. Adrenalectomized rats received drinking water containing 1% sodium chloride and were employed in test procedures 24 and 48 hours after surgery. Hypophysectomized animals were tested 36 and 60 hours following surgery. Adrenal demedullated rats received water containing 1% sodium chloride and 5% glucose for 7 days following surgery, and tapwater thereafter. The animals were tested 21 days following demedullation.

Animals were "gentled" by daily handling and intraperitoneal injections of 0.9% sodium chloride solution (NSS) to avoid excessive, handling-induced, pituitary-adrenal discharge. Sacrifice was by cervical dislocation.

The adrenal glands were removed, trimmed of fat and weighed, homogenized in a glass homogenizer in approximately 4 ml of 5% trichloroacetic acid at 4°C, and centrifuged at 2000 rpm for 5 minutes. Determinations of ascorbic acid concentration were performed with the supernate using the colorimetric method of Maickel(12).

The concentration of LNPSH was determined using the amperometric titration procedure of Benesch and Benesch(13). A single lobe of the liver was excised and frozen on dry ice immediately after sacrifice. Determinations of LNPSH were made within 24 hours. Because of reports of diurnal variation in liver glutathione(14), a group of NSS treated controls was included in each experiment for each time interval studied.

General procedures. Chlorpromazine hydrochloride§ aqueous solutions were administered intraperitoneally. Corticotropin¶ (ACTH) solutions were made using NSS and were administered *via* the lateral tail vein. Corticosterone‖ was administered intraperitoneally in 10% v/v alcoholic (U.S.P.) NSS solution. Corticosterone was first solubilized in the alcohol and then diluted to volume with NSS. Commercially available epinephrine hydrochloride,** 1:1,000, was diluted with NSS to 1:10,000. Administration was by subcutaneous injection.

Unilateral hindleg ligation was performed using rubber bands(7,15). Ligatures remained in position for 2½ hours and then were removed.

The Student "T" test of significance was employed to detect statistical difference. A probability level of 0.05 was considered statistically significant.

* This study was supported in part by Grant MY-2405, N.I.M.H., and Am. Foundation for Pharmaceutical Education.

† Present address: Procter & Gamble Co., Ivorydale Technical Center, Cincinnati, Ohio.

‡ Present address: Pitman-Moore Div., Dow Chemical Co., Indianapolis, Ind.

§ Generously supplied by Smith Kline and French Laboratories, Philadelphia.

¶ ACTHAR, Armour Pharmaceutical Co., Kankakee, Ill.

‖ Calbiochem, Los Angeles, Calif.

** Adrenalin Chloride Solution, Parke, Davis & Co., Detroit, Mich.

Results. Administration of 10 mg/kg of chlorpromazine HCl resulted in significant depletion (<0.02) of LNPSH 4 hours after the treatment. LNPSH levels did not differ from control levels at ½, 2, 8 and 20 hours after dosing (Fig. 1a). Diurnal variation in

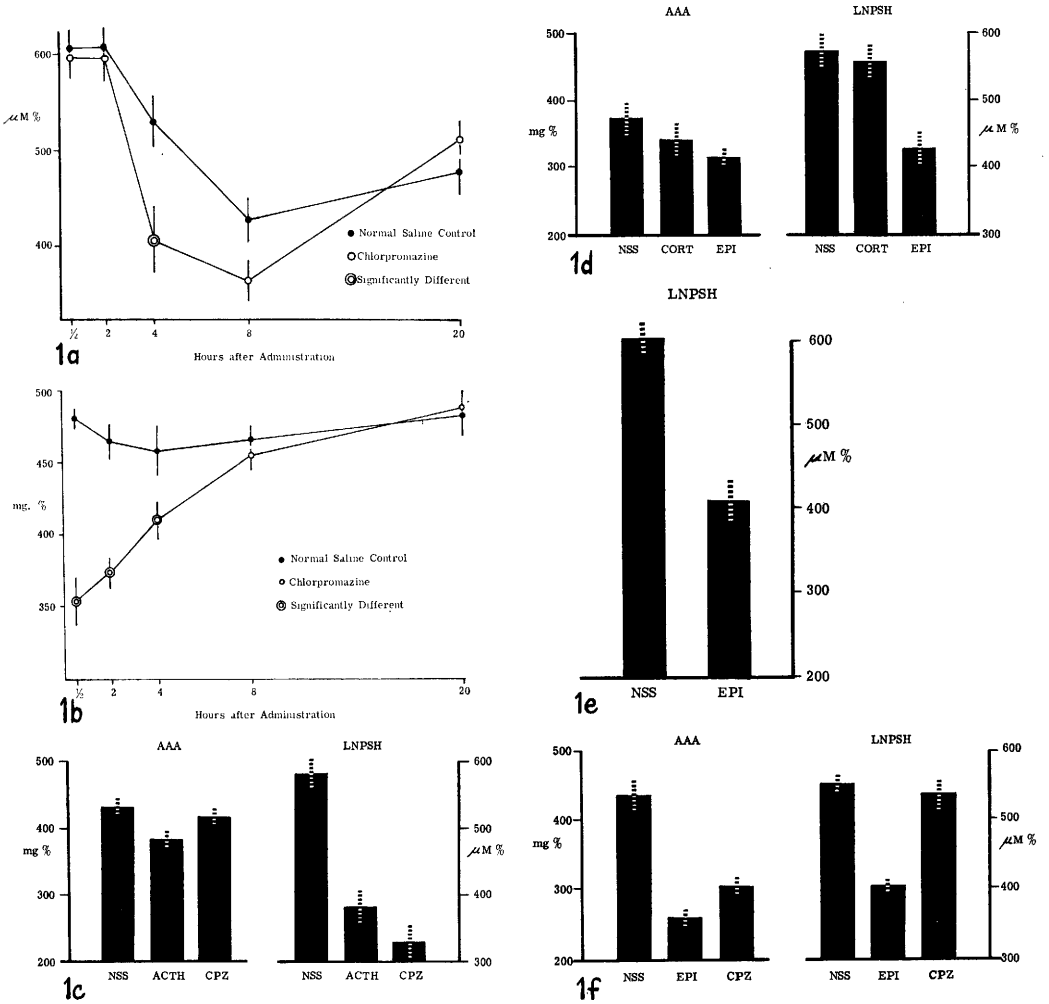

FIG. 1. Effects of several treatments on liver nonprotein sulfhydryl, LNPSH, and adrenal ascorbic acid, AAA, levels in the rat. Each plot based upon groups of 6 rats each unless stated otherwise. Drugs administered in aqueous solution. Standard errors of means are indicated by solid or broken vertical lines; significance level 0.05.

FIG. 1a. Effect of 10 mg/kg of chlorpromazine, CPZ, intraperitoneally (intraper.) on LNPSH concentrations. Data for 8 hr, and 20 hr control plots, and 20 hr CPZ plot based upon groups of 5 rats each.

FIG. 1b. Effect of 10 mg/kg of CPZ (intraper.) on AAA concentrations in the animals employed for Fig. 1a. Data for 20 hr CPZ based upon 5 rats.

FIG. 1c. Effects of 10 mg/kg of CPZ (intraper.) or 100 mu of ACTH intravenously (intraven.) on LNPSH and AAA concentrations in hypophysectomized rats.

FIG. 1d. Effects of 50 μg of corticosterone, CORT, (intraper.) or divided doses of 1:10,000 epinephrine, EPI, subcutaneously (subcutan.; 90 μg total dose) on LNPSH and AAA in hypophysectomized rats.

FIG. 1e. Effect of divided doses of 1:10,000 EPI (subcutan.; 450 μg total dose) on LNPSH in corticosterone-maintained (intraper.) adrenalectomized rats. Data for 0.9% sodium chloride, NSS, based upon 5 rats.

FIG. 1f. Effects of 10 mg/kg of CPZ (intraper.) or divided doses of 1:10,000 EPI (subcutan.; 450 μg total dose) on LNPSH and AAA in adrenal demedullated rats.

LNPSH levels is also apparent in this Figure. In comparison, adrenal ascorbic acid (AAA) was depleted at ½ (<0.001), 2 (<0.01) and 4 (<0.05) hours after administration, but not at 8 or 20 hours after administration (Fig. 1b).

Whether the hypophysis is necessary for CPZ-induced LNPSH depletion was determined. Hypophysectomized rats†† were treated with 10 mg/kg of chlorpromazine HCl, 100 milliunits of ACTH, or NSS, 36 hours post hypophysectomy. Four hours after administration, both AAA and LNPSH were significantly lower (<0.01) in the ACTH-treated rats as compared with control animals. The chlorpromazine-treated rats displayed a depletion of LNPSH (<0.001) but not of AAA (Fig. 1c).

In a similar experiment performed after hypophysectomy 3 groups of rats were given subcutaneous doses of 30 μg of epinephrine HCl at 4, 3, and 2 hours before sacrifice, and single, intraperitoneal doses of 50 μg of corticosterone or NSS at 4 hours before sacrifice. Corticosterone had no effect upon AAA or LNPSH; whereas, epinephrine evoked a significant depletion of both AAA (<0.05) and LNPSH (<0.001) (Fig. 1d).

Three groups of 6 rats each were adrenalectomized, employing the dorsal approach, 48 hours before administration of the test drugs. Corticosterone (50 μg) was administered to one group 20 and 5 hours prior to sacrifice. The remaining 2 groups of rats received chlorpromazine, or equivalent volumes of NSS, respectively. Chlorpromazine failed to induce a depletion of LNPSH.

Subsequently, 2 groups of 6 rats each were adrenalectomized and were given 50 μg of corticosterone immediately following the adrenalectomy, and 5 hours before sacrifice. One group received one milliliter of 1:10,000 epinephrine solution 4 hours before sacrifice, and one-half milliliter every one-half hour thereafter. The final epinephrine injection was made one-half hour before sacrifice. The remaining group received NSS according to the schedule observed for epinephrine. Epinephrine administration effected a significant decrease in LNPSH (<0.001) (Fig. 1e).

†† Hormone Assay Laboratories, Inc., Chicago, Ill.

Beck and Rieck(16) presented evidence that the adrenal cortex and the sympathetic nervous system, but not the adrenal medulla, are essential for trauma-induced depletion of LNPSH in the male mouse. Accordingly, administration of chlorpromazine to adrenal demedullated rats should induce a depletion of LNPSH—provided the mechanism of CPZ-induced LNPSH depletion in the female rat is comparable with the trauma-induced LNPSH depletion in the mouse.

Three groups of 6 rats each were adrenal demedullated†† 21 days prior to test procedures. One group received chlorpromazine, a second, epinephrine according to the above dosage schedule, and a third group, subcutaneous injections of NSS. Four hours following administration of CPZ, and the initial injections of epinephrine and NSS the animals were sacrificed and the adrenals and liver samples taken as previously described. Both CPZ and epinephrine treatments reduced AAA (<0.001); whereas, only epinephrine depleted LNPSH (<0.01) (Fig. 1f).

Discussion. From the results of this and other studies it would appear that the hypophyseal hormones are not essential factors in reactions which lead to the lowering of LNPSH. Although ACTH injection resulted in decreased LNPSH, as well as AAA, chlorpromazine and epinephrine effectively lowered LNPSH levels in the absence of the pituitary.

The presence of adrenal corticosteroids appears to be necessary for the CPZ-induced depletion of LNPSH. LNPSH depletion occurred only in rats with functioning adrenal cortices, or in corticosterone-maintained, adrenalectomized rats. However, in view of the inability of corticosterone to deplete LNPSH or AAA, it is unlikely that it is the immediate activator of the depletion mechanism. The report of Mennear *et al*(5) which described the prevention of LNPSH depletion following pretreatment with amphenone B (which inhibits secretion of corticosterone) agrees with this contention. On the other hand, epinephrine but not CPZ depleted LNPSH in adrenal demedullated rats as well as in adrenalectomized, corticosterone-maintained rats.

The present experiments have produced evi-

dence that in the female rat both the adrenal cortex and the adrenal medulla are essential for CPZ-induced LNPSH depletion. It is possible that any discrepancy between the evidence of Beck and Rieck(16), and the apparent necessity for a functioning adrenal medulla inferred herein may be a result of the extreme degree of stress from bilateral hindleg ligation employed by them as compared with the relatively mild stress induced by CPZ. Nagakura(17) hypothesized that severe stress can effect the release of relatively large quantities of extra-medullary catecholamines. Consequently, catecholamine release, but not necessarily a functioning adrenal medulla, may be responsible for LNPSH depletion. This is consistent with the results of preliminary experiments in our laboratories which indicate that the LNPSH lowering effect of CPZ can be prevented by pretreatment with CPZ for 5 days. Hindleg ligation-induced lowering of LNPSH was not affected with the same pretreatment.

Summary and conclusions. CPZ induced a depletion of LNPSH which differed significantly from control levels at 4 hours, but not at ½, 2, 8 and 20 hours after administration. The adrenal cortex, but not the hypophysis are integral components of the depleting mechanism. The release of epinephrine in the presence of corticosteroids may be the mediator of the CPZ-induced LNPSH depletion.

1. Bartlett, R. G., Register, U. D., Proc. Soc. Exp. Biol. and Med., 1953, v83, 708.
2. Bartlett, R. G., Jr., Register, U. D., ibid., 1955, v90, 500.
3. Beck, L. V., Linkenheimer, W., ibid., 1952, v81, 291.
4. Bradley, C. A., Miya, T. S., Yim, G. K. W., J. Neuropsychiat., 1961, v2, 175.
5. Mennear, J. H., Bradley, C. A., Miya, T. S., Proc. Soc. Exp. Biol. and Med., 1963, v112, 199.
6. Register, U. D., Bartlett, R. G., Jr., Science, 1954, v120, 109.
7. Takesue, E. I., Miya, T. S., J. Am. Pharm. Assn., Sci. Ed., 1957, v46, 19.
8. Wilson, J. E., Roberts, N., Beck, L. V., Fed. Proc., 1963, v22, Suppl. 2, 237.
9. Harkins, H. N., Physiol. Rev., 1945, v25, 531.
10. Sayers, G., Sayers, M. A., Endocrinology, 1947, v40, 265.
11. Sayers, G., Sayers, M. A., Liang, T.-Y., Long, C. N. H., 1945, v37, 96.
12. Maickel, R. P., Anal. Biochem., 1960, v1, 498.
13. Benesch, R. E., Benesch, R., Arch. Biochem., 1950, v28, 43.
14. Beck, L. V., Rieck, V. D., Duncan, B., Proc. Soc. Exp. Biol. and Med., 1958, v97, 229.
15. Rosenthal, S. M., U. S. Pub. Health Rep., 1943, v57, 1923.
16. Beck, L. V., Rieck, V. R., Proc. Exp. Biol. and Med., 1958, v98, 466.
17. Nagakura, I., Tohuku J. Exp. Med., 1959, v50, 39, (Through Von Euler, U. S., Fed. Proc., 1960, v19, Suppl. 5, 79).

Received January 24, 1966. P.S.E.B.M., 1966, v122.

High Specific Activity Labeling of Protein with I^{131} by the Iodine Monochloride Method.* (31148)

W. F. BALE, R. W. HELMKAMP, T. P. DAVIS, M. J. IZZO, R. L. GOODLAND, M. A. CONTRERAS AND I. L. SPAR

Department of Radiation Biology and Biophysics, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

Different lines of medical research and development make simple techniques desirable for attaching radioactive iodine isotopes to protein at radioactivity levels that may range for I^{131} as high as 300 mc per milligram protein. In many cases it is also essential that the methods used should result in minimal alteration of the protein either through radiation damage or by coupling an excessive amount of iodine (radioactive and non-radioactive) to the protein(1). This report describes further developments in this

*This work was performed under contract with U. S. Atomic Energy Commission.