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A Study of the Chlorpromazine Induced Decreased Liver Glutathione Reaction.* (27991)

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Bartlett and Register(1) reported that administration of chlorpromazine to rats induced a lowering of liver non-protein sulfhydryl (NPSH). The decreased liver NPSH reaction is also elicited by a number of nonspecific stressors(2-4). Register and Bartlett(5) have shown that injection of epinephrine into rats produced the decreased liver NPSH reaction. Earlier work in these laboratories(6) has shown that in addition to epinephrine, administration of the psychotomimetic agents lysergic acid diethylamide, mescaline and serotonin produces a lowering of rat liver NPSH. The major portion of total NPSH in tissue is composed of reduced glutathione and changes in tissue concentrations of NPSH are due to changes in concentrations of reduced glutathione(7).

This investigation concerns the mechanism through which chlorpromazine decreases liver glutathione.

Methods. Determination of liver concentrations of glutathione was made by the amperometric titration procedure of Benesch and Benesch(8) employed in our laboratories(4,6, 9 and 10).

One group of 10 female Holtzman rats (150-200 g) received intraperitoneal injections of 10 mg/kg of chlorpromazine while 2 other groups received saline. Twenty-four hours after the first injections the chlorpromazine treated rats received a second injection of 10 mg/kg of chlorpromazine. The 2 remaining groups were administered 10 mg/kg of chlorpromazine and an equal volume of saline, respectively. The rats were sacrificed 4 hours after the final injection for liver glutathione determination. In a second experiment 6 rats were injected with 10 mg/kg of chlorpromazine while a second group received saline. The rats were sacrificed 8 hours after the injections and liver glutathione levels determined.

Rats adrenalectomized 24 hours prior to the experiment were maintained at room temperature, received standard Wayne Lab Blox and 1% sodium chloride *ad libitum*. Twelve rats received 10 mg/kg of chlorpromazine and 10 received saline. Four hours after the injections the rats were sacrificed for liver glutathione determinations.

Hypophysectomized rats§ were used immediately upon receipt and required no special treatment. Approximately 8 hours after surgery 6 of the rats received injections of 10 mg/kg of chlorpromazine and a second group of operated rats were subjected to bilateral hind leg rubber band tourniquet stress. The tourniquets were removed after 3½ hours. The rats were sacrificed 4 hours after administration of chlorpromazine or application of the tourniquets.

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§ From Hormone Assay Laboratories, Inc., Chicago, Ill.

TABLE I. Liver Glutathione Levels of Rats after Various Treatments.

	Treatment	No. of rats	Mean liver GSH, μM % ± SE	% change from control	P
A	Control	10	503 ± 10		
	Chlorpromazine (1 dose)	"	417 ± 8	-17.09	<.001
	" (2 doses)	"	498 ± 8	- .01	ns
B	Control	6	403 ± 9		
	Chlorpromazine (8 hr prior to sacrifice)	"	408 ± 9	+ .01	ns
C*	Adrenex control	10	693 ± 10		
	" chlorpromazine	12	677 ± 10	- 2.3	ns
D†	Normal control	6	563 ± 7		
	Hypox "	"	557 ± 4	- 1.0	ns
	" chlorpromazine	"	465 ± 8	-20.1	<.001
	" stress	"	487 ± 16	-12.6	<.01
E‡	Control	10	486 ± 6		
	Chlorpromazine	"	391 ± 14	-19.5	<.001
	Amphenone-B	"	479 ± 8	- 1.4	ns
	" , chlorpromazine	"	487 ± 9	+ .2	ns
F§	Adrenex control	6	632 ± 11		
	" epinephrine	"	543 ± 13	-14.1	<.01
G§	Adrenex control	4	496 ± 18		
	" chlorpromazine	"	515 ± 9	+ 3.7	ns

* Adrenalectomized 24 hr prior to chlorpromazine administration and 28 hr prior to sacrifice.
† Hypophysectomized 8 hr prior to chlorpromazine or stress and 12 hr prior to sacrifice.
‡ Amphenone-B administered 8 hr prior to chlorpromazine and 12 hr prior to sacrifice.
§ Adrenalectomized and maintained on hydrocortisone (see text).

Amphenone-B‖ (3,3-Bis p-aminophenyl-2-butanone), 100 mg/kg ip, was administered to 10 rats 8 hours prior to injection of chlorpromazine. A second group received injections of Amphenone-B alone, while another group received only chlorpromazine. The rats were sacrificed 4 hours after injection of chlorpromazine (12 hours after Amphenone-B) and liver glutathione levels determined.

Immediately after adrenalectomy, 12 rats received 2.5 mg/kg, sc, of hydrocortisone. Twenty-four hours after surgery a second injection of hydrocortisone was made. One hour after the second injection of hydrocortisone 500 μg of epinephrine were administered in divided doses as described previously(6). Thirty minutes after the final injection the rats were sacrificed and liver glutathione levels determined.

In a similar experiment, hydrocortisone-maintained, adrenalectomized rats received injections of chlorpromazine. Four hours later the rats were sacrificed for liver glutathione determination.

Results. The results summarized in section A, Table I, show that as reported by Bartlett and Register(1) administration of a single dose of chlorpromazine induces a significant lowering of liver glutathione (417 μM% *vs* 503 μM%, t_{18}-4.65, $p < 0.001$) which returns to normal 8 hours after administration of chlorpromazine (403 μM% *vs* 408 μM%) (Table I-B). When 2 doses of chlorpromazine are administered 24 hours apart, the second injection does not induce lowering of liver glutathione (498 μM% *vs* 503 μM%) (Table I-A).

The results in Table I-C show that adrenalectomy blocks the liver glutathione lowering activity of a single injection of chlorpromazine. No significant difference was detected between liver glutathione levels of the adrenalectomized control rats and the chlorpromazine injected adrenalectomized rats (693 μM% *vs* 677 μM%).

The results in Table I-D indicate that hypophysectomy has no effect on liver glutathione levels (563 μM% *vs* 557 μM%). Injection of chlorpromazine into hypophysectomized rats induced a lowering of liver glutathione (465 μM% *vs* 557 μM%, $t_{10} = 7.67$, $p < 0.001$). Exposure to stress also lowered

‖ Supplied by Ciba Pharmaceutical Products, Summit, N. J.

liver glutathione levels of operated rats (487 μM% *vs* 557 μM%, $t_{10} = 3.48$, $p < 0.01$).

Administration of 100 mg/kg of Amphenone-B 8 hr prior to administration of chlorpromazine is effective in preventing the decreased liver glutathione reaction (487 μM% *vs* 479 μM%) (Table I-E). Chlorpromazine significantly lowered liver glutathione levels in the non-pretreated rats (391 μM% *vs* 486 μM%, $t_{18} = 6.25$, $p < 0.001$). Administration of Amphenone-B to rats 21 hours prior to sacrifice had no effect on liver glutathione levels (479 μM% *vs* 486 μM%).

The results in Section F, Table I, show that administration of epinephrine to hydrocortisone-maintained, adrenalectomized rats resulted in lowering of liver glutathione levels (543 μM% *vs* 632 μM%, $t_{10} = 3.72$, $p < 0.01$). Administration of chlorpromazine to hydrocortisone-maintained, adrenalectomized rats had no effect on liver glutathione (515 μM% *vs* 496 μM%) (Section G, Table I).

Discussion. The uncertainties surrounding the biological role(s) of glutathione make it difficult to assess the biological significance of the data. The results, however, help to explain the mechanism of the decreased liver glutathione reaction in response to chlorpromazine.

Beck and Rieck(11) suggested that the decreased liver NPSH reaction in response to stress is dependent upon the integrity of the adrenal cortical and sympathetic nervous systems. Our results show that the response to administration of chlorpromazine requires both the adrenal cortex and medulla. Beck and Rieck(11) reported that administration of epinephrine to adrenalectomized mice did not lower liver NPSH. This finding was confirmed by us in preliminary experiments, and further, it was demonstrated that administration of epinephrine to hydrocortisone-maintained, adrenalectomized rats lowered liver glutathione. Amphenone-B has been shown to inhibit secretion of adrenal corticoids(12). Although blood levels of corticoids were not determined during this study, it is assumed that they were reduced significantly after administration of Amphenone-B. The failure of chlorpromazine to lower liver glutathione in the Amphenone-B treated rats further points to the involvement of the cortical hormones in the reaction. The inability of chlorpromazine to lower liver glutathione in the hydrocortisone-maintained adrenalectomized rat indicates a requirement for release of epinephrine in response to administration of chlorpromazine. Furthermore, we have shown in our laboratories that pretreatment with the adrenergic blocking drug Dibenamine (10 mg/kg) prevents the chlorpromazine induced decreased liver glutathione reaction. There appears to be a threshold level of corticoids in the blood which must be maintained for production of the decreased liver glutathione reaction. Although administration of glucocorticoids to the intact rat produces the decreased liver glutathione reaction(13) it has been our experience that relatively high doses are required. Possibly the normal blood levels of the steroids function to sensitize the liver to the glutathione lowering effects of epinephrine.

The failure of hypophysectomy to prevent the response points to a possible involvement of aldosterone. Secretion of aldosterone is not controlled by the pituitary, and its secretion is increased in cases of stress.

Our results suggest that the initial fall in liver glutathione indicates a state of stress within the rat. The hypotensive or hypothermic effects of chlorpromazine could both be considered as stressors, either of which might be sufficient to initiate a discharge of epinephrine from the adrenal medulla.

The ability of a single injection of chlorpromazine to protect rats against the liver glutathione lowering effect of a second injection is an interesting phenomenon. Further investigation is under way.

Summary. The chlorpromazine-induced decrease in liver glutathione has been shown to be dependent on both adrenal medullary and cortical hormones, but not pituitary hormones.

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Separation of ACTH and MSH Fractions from Pituitaries of Different Species.* (27992)

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We have shown that ACTH evokes a specific melanophore reaction in frogs which cannot be ascribed to contamination by infinitesimal amounts of MSH(1-3). This view was also held by Johnsson and Högberg(4) but was vehemently contested by Li and his collaborators(5). Recently our view was vindicated after the amino-acid sequence of ACTH(6-9), alpha-MSH(10,11) and beta-MSH(12,13) was determined. These 3 hormones contain a core of 7 amino-acids:

1 2 3 4 5 6 7
(Met-Glu-His-Phe-Arg-Try-Gly)

which elicits the melanophore effect both in man and frogs(6).

Thus, 3 melanophore hormones exist in the pituitary, one in the anterior lobe (ACTH), one in the intermediate lobe (alpha-MSH or intermedin), one in the posterior lobe (beta-MSH), which were separated by us biologically(14,15).

The present paper deals with the fractionation and chromatographic separation of the 3 melanophore hormones from pituitaries of 3 different species: oxen, sheep and pigs.

Materials and methods. Separation of ACTH, alpha-MSH and beta-MSH was carried out according to the method of Steelman *et al.*(17). The starting material was a concentrate obtained by purifying dry powders of the anterior and posterior lobes of the pituitary glands of oxen, sheep and pigs. These concentrates were partially purified by the Landgrebe and Mitchell method(18) after adsorption of ACTH with oxycellulose. Four ml concentrates were dissolved in 100 ml distilled water adjusted to pH 4 with 10% hydrochloric acid, and dialyzed for 24 hours against distilled water. The dialyzed solution was adjusted to pH 5.8 with 0.1 M ammonium hydroxide and centrifuged. The clear supernatant was placed on a column (12 × 400 mm) with sodium carboxy methyl cellulose (CMC) absorbent (0.4-0.5 meq./g).

The CMC was prepared by reaction of monochloroacetic acid with a suspension of cellulose in concentrated sodium hydroxide, then equilibrated with 0.01 M ammonium acetate (pH 5.8) after washing with about 400 ml 0.01 M ammonium acetate. Beta-MSH was obtained first by a fraction collector in 100 tubes containing 3 ml samples, alpha-MSH being retained on the column. Elution of alpha-MSH was accomplished at a gradient of 0.1 M ammonium acetate of pH 6.5 passing through a mixing chamber of 200 ml of 0.01 M ammonium acetate buffer. The alpha-MSH was obtained through the fraction collector in another 100 tubes.

The peptides were identified in the tubes by the method of Anderson *et al.*(19) with a

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