Role of the Pituitary and Adrenals in Cold and Restraint Induced Liver Nonprotein Sulfhydryl Depletion.* (31721)

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A number of investigators have studied liver nonprotein sulfhydryl levels (LNPSH) in relation to various forms of stress. Decreases in LNPSH have been demonstrated in association with severe trauma such as tumbling or scalding(1,2), cold and restraint (2-4) or injection of bacterial polysaccharides (2). Various pharmacologic agents cause a similar effect. These include insulin(5,6), ACTH(7), epinephrine(8), chlorpromazine (9), lysergic acid diethylamide, mescaline and serotonin creatinine sulfate(10).

This paper is concerned with the role of the pituitary and adrenals in the cold and restraint induced depletion of LNPSH.

Experimental methods. The investigation was divided into two major sections. The first sought to elucidate the role of the pituitary and the second the adrenals. In the first experiment 83 female Sprague-Dawley rats (150-180 g), 57 of which were hypophysectomized,† were divided into 12 groups as outlined in Table I.

The second experiment utilized 62 female rats (170-200 g) divided into 8 groups as outlined in Table II.

Adrenalectomy was performed using the technique of D'Amour and Blood(11) one week prior to stressing. During this time the rats were maintained on stock diet, water, and saline.

The stressing procedure employed was that described by Register and Bartlett(4). The rats were placed in loose fitting restraining cages and cooled in a refrigerated room (0-5°C) so that their rectal temperatures were decreased 20°C over a 4-hour period in 2° to 3° increments.

Immediately after this stress the animals were decapitated and their livers removed and frozen in dry ice. LNPSH was determined using the modified amperometric titration method of Benesch *et al*(12) with the rotating platinum electrode assembly described by Agazzi *et al*(13). Those animals to be adapted to restraint were kept in the loose fitting cages for 5 days at room temperature with food and water provided.

The adapted hypophysectomized animals were given 1 mg ACTH twice daily for 4 days and the non-adapted a similar dosage for 2 days prior to the stress day. On the day of stress they received a second ACTH injection 2 hours after the start of the cooling period. Where indicated epinephrine was given as a 1:10,000 solution of adrenalin hydrochloride, 1 ml initially then 0.5 ml every half hour for 4½ hours just prior to sacrificing. When cortisol was used 0.2 mg was given daily subcutaneously for 7 days before stress.

Results. A. Hypophysectomized animals. The stress of cold and restraint resulted in a significant ($P<.01$) depletion of LNPSH in all groups tested-intact, hypophysectomized and hypophysectomized given ACTH. In the stressed animals the LNPSH was significantly lower in the hypophysectomized ($P<.05$) and hypophysectomized given ACTH ($P<.01$) when compared with the intact stressed animals. This difference between groups was not seen in the animals adapted to restraint prior to cold stress. This might be explained by assuming that the restraint was a greater immediate stress on the hypophysectomized animals, but that this difference was overcome by the adaptation period.

Both the intact and hypophysectomized animals demonstrated significant ($P<.01$) decreases in LNPSH after injection with epinephrine. However the hypophysectomized animals did not show as great a decrease in LNPSH as the intact controls.

B. Adrenalectomized animals. All animals in this group showed a significant ($P<.01$) depletion of LNPSH following cold and

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† Hypophysectomized rats were obtained from Hormone Assay Laboratories, Inc., Chicago, Ill.

TABLE I. LNPSH Changes in Response to Cold and Restraint, and Epinephrine in Intact and Hypophysectomized Rats.

	NPSH, μmoles/g liver tissue		
	Intact	Hypophysectomized	Hypophysectomized + ACTH
Controls	7.8 ± .27 (7)	7.9 ± .24*(13)	7.0 ± .24 (6)†
Stressed	3.8 ± .23 (6)	2.9 ± .32 (6)	2.6 ± .12 (7)
Restraint adapted—stressed	3.5 ± .14 (6)	3.4 ± .42 (5)	4.1 ± .35 (7)
" " —not stressed			7.3 ± .27 (6)
Injected with epinephrine	3.3 ± .09 (7)	5.3 ± .09 (7)	

* Mean ± standard error of mean. † No. of rats in each group.

restraint. This decrease was observed whether the adrenalectomized animals had been maintained on cortisol or not.

Epinephrine injection decreased LNPSH ($P<.01$) in adrenalectomized animals and this was enhanced by simultaneous cold and restraint.

Discussion. From the data presented it appears that the presence of the pituitary is not necessary for cold and restraint to decrease LNPSH. This agrees with similar results obtained by Weaver *et al*(9) using chlorpromazine as the stressing agent.

When injected with epinephrine, intact and hypophysectomized rats both showed significant decreases in LNPSH although the intact animals demonstrated the greater depletion. This could be related to an inability of the hypophysectomized animals to produce ACTH which has been shown to reduce LNPSH(7). ACTH can produce hypoglycemia even in adrenalectomized animals (14) and this could act as a stressing agent in a similar manner to insulin(5,6). Since both groups were tested simultaneously the diurnal variation of LNPSH described by Beck(15) would not account for the differences noted.

TABLE II. Effect of Cold and Restraint on LNPSH in Adrenalectomized Rats.

	NPSH, μmoles/g liver tissue	
	Unstressed	Stressed
Intact	5.40 ±.17*(8)	2.42 ±.12 (7)†
Adrenalectomized	4.90 ±.16 (7)	2.39 ±.14 (8)
Adrenalectomized given cortisol	4.69 ±.27 (8)	2.63 ±.33 (8)
Adrenalectomized given epinephrine	3.26 ±.16 (7)	2.25 ±.11 (8)

* Mean ± standard error of mean.
† No. of rats in each group.

Using cold and restraint as the stressing agent a significant decrease in LNPSH was observed in adrenalectomized animals. However other investigators using trauma(16) and chlorpromazine(9) suggested that a functioning adrenal cortex was necessary for the lowering of LNPSH. Mennear *et al* also reported that treatment with amphenone B, which inhibits adrenal cortical secretion blocked the chlorpromazine induced LNPSH depletion(17). The stress of cold and restraint used in our study may have been more severe than chlorpromazine or tourniquet trauma or may act through different pathways to decrease LNPSH.

Weaver *et al*(9) reported a significant decrease in LNPSH after epinephrine administration in adrenalectomized animals maintained on cortisol. In the present study this decrease in LNPSH was observed not only in the cortisol maintained animals given epinephrine or subjected to cold and restraint but also in adrenalectomized animals not on cortisol.

It has been suggested that the sympathetic nervous system also plays an important role in LNPSH regulation as LNPSH depletion from stress has been blocked by a ganglionic blocking agent(16). The present data suggest that stimulation of the sympathetic nervous system by cold and restraint may deplete LNPSH in the absence of either the pituitary or adrenals.

Summary. A significant decrease in liver nonprotein sulfhydryl levels (LNPSH) was demonstrated in intact, hypophysectomized and adrenalectomized female rats in response to cold and restraint. A similar response was demonstrated following epinephrine injection. Neither ACTH nor cortisol replacement was

necessary for this response. It is concluded that maximum LNPSH depletion during cold and restraint can be effected through stimulation of the sympathetic nervous system although other mechanisms are operative in intact animals.

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Evidence for Omitting the Petroleum Ether Extraction in Plasma Corticosterone Determination.* (31722)

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The original method for spectrophotofluorimetric determination of serum corticoids(1) and its modification(2) suggest that the sample be extracted with either pertoleum ether or isooctane. This step removes neutral lipids(3) which would interfere with the analysis of the corticoids. However, the studies of Callard *et al*(4) indicate that 20 α-hydroxy-pregn-4-en-3-one (hereafter called 20 α-hydroxyprogesterone) may be the main interfering fluorogen in determination of plasma corticosteroid levels. This compound has absorption and fluorescent spectra which are similar to corticosterone(4). Twenty α-hydroxyprogesterone has been found in the plasma and ovary of pregnant female rats(5) and in the venous effluent of the ovary in normal female rats(6). About half of this compound is removed from plasma by an extraction with petroleum ether (B.P. 40-60°) (4).

If only the ovary produces 20 α-hydroxyprogesterone and if it is the main source of error in the fluorimetric method for corticoid determination, then the extraction with petroleum ether may be unnecessary for the analysis of samples from male and ovariectomized female rats. The following experiments were designed to test this hypothesis.

Methods and materials. Fifty-nine Sprague-Dawley rats were sacrificed by guillotine or under ether. The blood was collected in heparinized containers and centrifuged at 1000 × *g*. The plasma was removed and frozen.

Of these 59 rats, 9 were males, intact or hypophysectomized. Nineteen were females which had been ovariectomized 2 weeks prior to use. Thirty-one animals were intact females which were maintained in a light-regu-

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