Inhibition of Hepatic Alanine Transaminase Activity in Response to Treatment with 11-Deoxycorticosterone.* (26856)

HOMER R. HARDING, FRED ROSEN AND CHARLES A. NICHOL
Department of Experimental Therapeutics, Roswell Park Memorial Institute, Buffalo, N. Y.

Alanine transaminase activity is increased markedly in the liver of rats treated with glucocorticoids or corticotropin(1-3). In contrast, treatment with deoxycorticosterone (2) or adrenalectomy(4) caused a significant reduction in level of this transaminase in liver. The stimulatory action of cortisol on hepatic alanine transaminase activity was not impaired when this steroid was given in combination with deoxycorticosterone(2). The purpose of this study was to determine whether the fall in liver alanine transaminase following administration of deoxycorticosterone is related to its inhibitory effect on release of corticotropin by the pituitary.

Materials and methods. Male hypophysectomized rats of the Sprague Dawley strain, obtained from the Charles River Laboratories were used. Rats were adrenalectomized 5 days prior to use and were given either 11-deoxycorticosterone or one per cent saline in place of drinking water. Deoxycorticosterone was administered subcutaneously as an aqueous suspension. Corticotropin (10 I.U. per injection, 20 I.U., total dose) (ACTHAR GEL, Armour Pharmaceutical Co.) was administered twice daily. Purina Laboratory Diet was fed *ad libitum* to all animals.

The animals were stunned by a blow on the head, then killed by exsanguination. The livers were immediately placed in beakers on crushed ice. Homogenates were prepared in a Waring Blendor with ice-cold distilled water. Alanine- and aspartate-α-ketoglutarate transaminase activities were measured by procedures(2) similar to those described by Wroblewski and La Due(5) except that instead of measuring the oxidation of DPNH spectrophotometrically, DPN was determined by a fluorometric procedure developed by Lowry and co-workers(6). Tyrosine-α-ketoglutarate transaminase activity was determined using a modification of the method described by Sereni and co-workers(7). Protein was determined by a modification of the Folin procedure(8).

Results. The data presented in Table I indicate that treatment of rats with deoxycorticosterone (DOC) causes a marked reduction in liver alanine transaminase activity. Administration of DOC to adult rats depressed the activity of this transaminase to 42% of control value. Treatment of younger animals with DOC also lowered the values of this transaminase in liver to 30% of normal.

The depression in hepatic alanine transaminase activity which followed adrenalectomy was comparable to that observed in unoperated rats treated with deoxycorticosterone (Table I). In contrast to the effect of DOC on alanine transaminase activity in intact animals, treatment of adrenalectomized rats with DOC for 14 days did not further reduce the activity of this enzyme in liver.

On the basis of these results, a direct effect of DOC on alanine transaminase appeared unlikely. The possibility that DOC might act by impairing the secretion of corticotropin by the hypophysis was tested by administration of DOC to hypophysectomized rats. Hepatic alanine transaminase values were 2 to 5 times higher in hypophysectomized rats than in intact rats of similar age and weight, whereas the values for tyrosine and aspartate transaminase in liver were not significantly altered by hypophysectomy (Table II). Treatment of hypophysectomized rats with DOC did not reduce the activity of alanine transaminase in liver nor did it affect the activity of either tyrosine or aspartate transaminase.

When hypophysectomized animals were treated with corticotropin, the activity of alanine and tyrosine transaminase in liver was

* Paper No. 5 in the series on Corticosteroids and Transaminase Activity. This work was supported in part by a research grant from Nat. Inst. of Arthritis and Metab. Dis.

TABLE I. Effects of Deoxycorticosterone and Adrenalectomy on Hepatic Alanine Transaminase Activity.

	Body wt (g)	Liver wt (g)	Alanine transaminase (mMoles of substrate utilized/hr) Per g wet wt	Per g protein
Intact, no treatment	407	14.14 ± .71*	2.79 ± .50	17.0 ± 2.8
" , 3 mg DOC daily for 7 days	374	11.03 ± .75	1.26 ± .42	7.2 ± 2.4
Intact, no treatment	195	8.82 ± 1.12	1.82 ± .41	10.7 ± 2.0
" , 3 mg DOC daily for 14 days	201	9.08 ± 1.22	.58 ± .23	3.27 ± 1.1
Adrenalectomized, no treatment	132	5.29 ± 1.23	.52 ± .23	3.20 ± .91
" , 3 mg DOC daily for 14 days	170	7.37 ± 1.06	.45 ± .26	2.44 ± 1.1

* Avg value of 5 or more rats/group ± stand. dev.

increased several-fold (Table II). Aspartate transaminase activity was not changed by administration of corticotropin. In hypophysectomized animals injected with both deoxycorticosterone and corticotropin, 2-fold increases in hepatic alanine and tyrosine transaminase activity were observed (Table II). An effect of DOC in suppressing the response to ACTH was questionable. In a similar experiment, however, corticotropin administered to hypophysectomized rats increased liver alanine transaminase activity to a value of 57.9 ± 7.0 mMoles of substrate per g protein per hour while treatment with both deoxycorticosterone and corticotropin gave a value of 52.4 ± 5.3. Thus, the response to treatment with both hormones was comparable to that obtained when corticotropin only was administered.

To insure that the effect of corticotropin on liver alanine transaminase activity was mediated through the adrenal gland, this hormone was administered to adrenalectomized rats. Treatment of adrenalectomized rats with 20 units of corticotropin daily for 7 days did not significantly alter liver alanine transaminase activity (Table II).

Discussion. Deoxycorticosterone appears to affect the activity of alanine transaminase of liver indirectly by interfering with release of corticotropin from the hypophysis. In support of this hypothesis is the failure of deoxycorticosterone to alter hepatic levels of alanine transaminase in adrenalectomized rats, in contrast to the marked decrease in the activity of this transaminase produced in unoperated animals by the same treatment. Previous studies indicated that deoxycorticosterone did not interfere with the response of alanine transaminase to cortisol(2), nor could the effect of deoxycorticosterone be attributed to loss or binding of pyridoxal phos-

TABLE II. Effect of Deoxycorticosterone and Corticotropin on Hepatic Transaminase Activity of Hypophysectomized Rats.

Treatment	Body wt (g)	Liver wt (g)	Transaminase activity (mMoles substrate utilized/g protein/hr) Alanine	Tyrosine	Aspartate
Intact	99	3.64 ± .26†	4.30 ± .97	.60 ± .15	127 ± 6
Hypophysectomized	102	3.78 ± .29	23.4 ± 1.2	.77 ± .10	95 ± 5
" + DOC*	93	3.97 ± .28	21.9 ± 2.5	.65 ± .26	114 ± 15
" + ACTH*	99	4.09 ± .30	58.3 ± 8.6	2.32 ± .47	149 ± 21
" + DOC + ACTH*	87	4.23 ± .69	40.8 ± 9.9	1.43 ± .64	103 ± 30
Adrenalectomized	150	4.58 ± .68	5.13 ± 1.4	—	—
" + ACTH*	174	6.36 ± .63	3.50 ± .45	—	—

* DOC = Deoxycorticosterone, 3 mg administered subcut. daily for 7 days. ACTH = Corticotropin, 10 units administered subcut. twice daily for 7 days.

† Avg value of 5 rats/group ± stand. dev.

phate, the coenzyme of alanine transaminase.†

In previous studies in this laboratory corticotropin increased hepatic alanine transaminase activity of intact rats(3). The effect of corticotropin on this transaminase is most likely mediated through the adrenal gland since no alteration in enzyme activity was noted when corticotropin was administered to adrenalectomized animals (Table II). The slight reduction in alanine transaminase response observed when deoxycorticosterone was administered simultaneously with corticotropin was not statistically significant. Recently it has been reported that corticosterone may inhibit adrenal steroid production in the rat by acting directly on the adrenal gland(9). The results of the present work do not exclude the possibility that deoxycorticosterone may act in a similar manner.

The release of corticotropin by the pituitary following cold stress or unilateral adrenalectomy has been found to be inhibited by treatment with corticosteroids(10-12). Sayers and Sayers(10) and Long(13) observed that the depletion of adrenal ascorbic acid following stress could be prevented by previous treatment of animals with adrenal steroids. Recently several assays for corticotropin have been based on the inhibition of its release from the pituitary by treatment with a glucocorticoid(14,15). Deoxycorticosterone also blocks the secretion of corticotropin by the pituitary(11,12) but has only one-eighth the potency of cortisone in this respect(10). The results obtained with deoxycorticosterone have been variable(16-18). Route of administration of deoxycorticosterone or severity of the stress stimulus(19) appear to be important factors concerning the relative suppression of corticotropin output.

The finding that deoxycorticosterone lowered the activity of alanine transaminase in liver was of interest since other cortisol responsive tissues(20) or systems(21) are either refractory or show a weak glucocorticoid response after treatment with this mineral corticoid.

† F. Rosen and R. J. Milholland, unpublished data.

There are numerous reports concerned with the effect of corticosteroids on enzyme activity, among them the effects of cortisone and cortisol on enhancement of activity of several transaminases, such as tyrosine-(22, 23), tryptophan-(24,25), and alanine-α-ketoglutarate transaminase(1-3,26) and of tryptophan pyrrolase(27,28). Subsequent studies demonstrated that the high endogenous levels of activity of alanine transaminase in the liver of adult rats were dependent on presence of the adrenal gland(4). Deoxycorticosterone was found to depress the basal level of alanine transaminase in liver(2) but to be without effect on tryptophan pyrrolase(29).

While the activity of tryptophan pyrrolase has been reported to be decreased following adrenalectomy(28), the level of tyrosine transaminase in liver was not altered after adrenal ablation(7).

The changes in alanine transaminase activity induced by treatment with deoxycorticosterone or cortisol provide a new technic for studying the inter-relationships of these corticosteroids with other hormones and systems *in vivo*. Changes in activity of this transaminase may be correlated with the physiological effects of cortisol and deoxycorticosterone. For example, it has been noted that the growth inhibition of Walker carcinoma 256 in rats treated with cortisol is associated with a 10-fold rise in tumor alanine transaminase activity whereas slight stimulation of tumor growth and significant depression in transaminase activity occurred following administration of deoxycorticosterone(30). In the present study, measurement of hepatic alanine transaminase activity after treatment with deoxycorticosterone was used as the basis for demonstrating that this hormone suppresses corticotropin secretion by the pituitary.

Summary. Daily administration of DOC for short periods resulted in a 60 to 70% depression in liver alanine transaminase activity. Adrenalectomy also caused a decrease in activity of alanine transaminase, but no further reduction in the level of this enzyme was observed upon treatment with DOC. Treatment with ACTH resulted in an increase in alanine transaminase activity in hy-

pophysectomized rats, but not in adrenalectomized animals. Administration of DOC to hypophysectomized rats failed to lower alanine transaminase, nor did it alter the response of this enzyme to treatment with ACTH. The inhibitory effect of DOC on alanine transaminase activity appears to be due to suppression of ACTH release by the pituitary.

The authors are indebted to Mr. Louis Budnick and Miss Phyllis Roble for excellent assistance in this investigation.

1. Rosen, F., Roberts, N. R., Budnick, L. E., Nichol, C. A., *Science,* 1958, v127, 287.

2. Rosen, F., Roberts, N. R., Nichol, C. A., *J. Biol. Chem.,* 1959, v234, 476.

3. Rosen, F., Roberts, N. R., Budnick, L. E., Nichol, C. A., *Endocrinology,* 1959, v65, 256.

4. Harding, H. R., Rosen, F., Nichol, C. A., *Am. J. Physiol.,* 1961, v207, 271.

5. Wroblewski, F., La Due, J. S., PROC. SOC. EXP. BIOL. AND MED., 1956, v91, 569.

6. Lowry, O. H., Roberts, N. R., Kapphahn, J. I., *J. Biol. Chem.* 1957, v224, 1047.

7. Sereni, F., Kenney, F. T., Kretchmer, N., *ibid.,* 1959, v234, 609.

8. Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. I., *ibid.,* 1951, v193, 265.

9. Peron, F. G., Moncloa, F., Dorfman, R. I., *Endocrinology,* 1960, v67, 379.

10. Sayers, G., Sayers, M. A., *ibid.,* 1947, v40, 265.

11. Hodges, J. R., *Brit. J. Pharmacol.,* 1953, v8, 242.

12. ———, *J. Endocrin.,* 1955, v12, 152.

13. Long, C. N. H., *Fed. Proc.,* 1947, v6, 461.

14. Hamburger, C., *Acta Endocrinologica,* 1960, v35, 595.

15. Kendall, J. W., *Clin. Res.,* 1961, v9, 73.

16. Moya, F., Selye, H., PROC. SOC. EXP. BIOL. AND MED., 1948, v68, 529.

17. Gershberg, H., Fry, E. G., Brobeck, J. R., Long, C. N. H., *Yale J. Biol. Med.,* 1950, v23, 32.

18. Hall, C. E., Finerty, J. C., Hall, O., Hess, M., *Endocrinology,* 1951, v48, 591.

19. Buttle, G. A., Hodges, J. R., *Ciba Foundation Colloquia on Endocrinology,* 1953, v5, 147.

20. Niedermeier, W., Carmichael, E. B., PROC. SOC. EXP. BIOL. AND MED., 1960, v104, 779.

21. Blecher, M., White, A., *J. Biol. Chem.,* 1958, v233, 1161.

22. Lin, E. C. C., Knox, W. E., *Biochim. et Biophys. Acta,* 1957, v26, 85.

23. Lin, E. C. C., Rivlin, R. S., Knox, W. E., *Am. J. Physiol.,* 1959, v196, 303.

24. Lin, E. C. C., Civen, M., Knox, W. E., *J. Biol. Chem.,* 1958, v233, 1183.

25. Civen, M., Knox, W. E., *Science,* 1959, v129, 1672.

26. LaCroix, E., Leusen, I., *Exp. Med. and Surg.,* 1959, v17, 170.

27. Knox, W. E., *Brit. J. Exp. Pathol.,* 1951, v32, 462.

28. Knox, W. E., Auerbach, V. H., *J. Biol. Chem.,* 1955, v214, 307.

29. Thomson, J. F., Mikuta, E. T., *Endocrinology,* 1954, v55, 232.

30. Rosen, F., Budnick, L. E., Solomon, D. K., Nichol, C. A., *Cancer Res.,* 1961, v21, 620.

Received July 10, 1961. P.S.E.B.M., 1961, v108.

Diminished Adrenal Corticoid Response to Burn and ACTH in the Nephrectomized Dog.* (26857)

DONALD S. GANN, BRYANT KINGSBURY, WILLIAM R. DRUCKER AND RANDALL H. TRAVIS (Introduced by George Sayers)

Departments of Surgery, Medicine and Physiology, Western Reserve University School of Medicine and University Hospitals of Cleveland, Ohio

Recent reports have implicated the kidney in the control of aldosterone secretion(1,2,3), and have suggested that this organ may play some role in control of 17 hydroxycorticoid (17 OHCS) secretion as well(3,4). Since ACTH is known to affect both aldosterone and 17 OHCS secretion, the present studies were undertaken to determine whether nephrectomy alters the 17 OHCS response to burn, a known stimulus to ACTH release, and if so, by what means.

* Supported in part by grants from Cleveland Area Heart Society and Nat. Inst. Health.

Methods. Experiments were performed on