Effect of ACTH on Distribution of Ascorbic Acid in Rat Adrenal Gland as Determined by Direct Microchemical Analysis

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Introduction

Since the discovery that the ascorbic acid content of the adrenal gland changes with adrenal stimulation, this fact has been widely used both to assess stress reactions in experimental animals and as a means of assaying ACTH. (1) By measuring total adrenal ascorbic acid it has been found that the amount of this substance falls rapidly after acute stress or ACTH administration, reaching a minimum in one to three hours (falling to about 40% of original value in rats). (1)

The distribution of ascorbic acid in the rat adrenal has been extensively investigated by Greep and Deane (2) and by Deane and Morse (3). They used a histochemical technique dependent on reduction of silver ion by ascorbic acid, Their results show silver-reducing material in all zones of the gland, with a fall following a single dose of ACTH limited to the zona fasciculata and reticularis. After prolonged stress (starvation) the silver-reducing material seemed normal in amount.

The limits of this histochemical technique are 1)that it is qualitative rather than quantitative, and 2) that one cannot be sure that the position of the ascorbic acid or the finally visualized material has not changed during the procedure. The present study was undertaken to determine quantitatively by direct microchemical analysis of adrenal slices the distribution of ascorbic acid in the various zones of the rat adrenal gland and to determine changes after ACTH administration.

Methods

The general method employed was the Linderstrøm-Lang technique as modified by Lowry (4). The animals were rapidly killed by decapitation, the adrenals promptly removed and dropped into liquid nitrogen. These were later mounted at a temperature of -20°C, and 20 μ section

of the frozen gland were cut. These were dehydrated at low temperatures and finally the desired zone of the gland, which could be readily identified in frozen section was dissected out, transferred to a test tube, and chemical analysis for ascorbic acid, cholesterol* and protein carried out.

Ascorbic acid was determined by the dinitrophenylhydrazine method as adapted for small samples by Lowry, Lopez, and Bessey (5). Protein content of each section was used as a reference and was determined by the method of Lowry, Rosebrough, Farr, and Randall (6).

Experimental

Adult female rats weighing 215 to 270 gm. were used. One adrenal from each rat was analysed. There were 3 control animals; 2 others were sacrificed 30 minutes after a single intravenous dose of ACTH (1 mg.), 2 90minutes after the same dose and 1 animal sacrificed 36 hrs. after the last of eight 1 mg. doses of ACTH given over a 3-day period.

Results

The results are presented in detail in tables I through IV and plotted in the accompanying figure. The figure was constructed by plotting the average values obtained in all the animals of a given group, the vertical lines representing the standard error of the mean (S.E.) for each value.** As shown, the normal rat adrenal has the highest concentration of ascorbic acid in the zona fasciculata

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* The cholesterol studies were done by Mr. Wayne Albers of the Dept. of Pharmacology and are not reported here.

** S.E. was calculated by the following formula:

$$S.E. \quad \frac{Sd^2}{n(n-1)}$$

and only slightly less in the inner zona reticularis. Following acute stimulation with ACTH the ascorbic acid concentration falls rather uniformly in all zones of the cortex except the glomerulosa, where after 90 minutes there is a slight fall of questionable significance. This constancy of glomerulosa ascorbic acid re-emphasizes the probable independende of the rat zona glomerulosa from the pituitary.

After prolonged stimulation with ACTH the reticularis was almost unrecognizable as such in frozen section due to marked widening of the fasciculata. Sections taken from the inner fasciculata zone down to the medulla are plotted as F-R. It is seen that with chronic stimulation adrenal ascorbic acid concentration remains at normal levels in the fascibulata and possibly increases in the zone between the fasciculata and the medulla. (Note that only one animal was used for chronic stimulation.)

Discussion

The adrenal gland with its rather sharply demarcated zones has been found to be readily susceptible to Lowry's technique of direct chemical analysis of dissected tissue slices. The present study of the distribution of ascorbic acid in the rat adrenal has in general confirmed earlier work using histochemical techniques and has quantitated the results. The significance of the adrenal ascorbic acid changes with stress or ACTH have yet to be determined. There is no clear evidence of a specific role in steroid metabolism (7).

Using the method described above, Albers of the Dept. of Pharmacology has extended the study of the "microchemical anatomy" of the adrenal by "mapping out" cholesterol and various enzymes in the different zones of the gland.

Conclusion

1) Direct microchemical analysis of dissected slices of rat adrenal for ascorbic acid and protein is reported.

2) The distribution of ascorbic acid in the various zones of the adrenal is plotted and the results show the highest consentrations in the zona fascibulata and inner zona reticularis.

3) Following acute ACTH stimulation, the ascorbic acid concentration falls rather unfformly throughout the cortex with the exception of the glomerulosa where it remains approximately constant or falls slightly. With chronic stimulation by ACTH the fascibulata, broadens and maintains its ascorbic acid content while the area adjacent to the medulla shows increased concentration of this substance.

This work was done with Dr. O.H. Lowry and Mr. Wayne Albers of the Department of Pharmacology, Washington University School of Medicine.

References

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(6) Lowry, Rosebrough, Farr, and Randall, JBC 193 , 265, 1951.

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