EFFECTS OF FLAVIN ADENINE DINUCLEOTIDE ON THE FINE STRUCTURE AND GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY OF ADRENAL GLANDS OF RAT TREATED WITH DEXAMETHASONE PHOSPHATE

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(Received September 25, 1973)

The determination of adrenal glucose-6-phosphate dehydrogenase (G-6-PD) activity and electron microscopic observations of the adrenals of rats treated simultaneously with dexamethasone phosphate and flavin adenine dinucleotide (FAD) were carried out in order to clarify one of the preventive mechanisms of FAD on steroid-induced adrenal atrophy. The result showed that decrease of adrenal G-6-PD activity was prevented by simultaneous administration of FAD and dexamethasone phosphate. Electron microscopy of the fascicular zone of the adrenals of rats suggests that their function is depressed by dexamethasone phosphate alone but not by dexamethasone phosphate plus FAD. It is thought that the preventive effects of FAD on the steroid-induced adrenal atrophy are closely related to the preservation of adrenal G-6-PD activity.

Recently, we demonstrated that flavin adenine dinucleotide (FAD) prevents the induction of adrenal atrophy by dexamethasone phosphate administration to a considerable degree (1). However, the mechanisms of action of FAD on the steroid-induced adrenal atrophy is still obscure.

In the present study, the fine structural studies of adrenals and determination of adrenal glucose-6-phosphate dehydrogenase (G-6-PD) (d-glucose-6-phosphate: NADP oxidoreductase, EC 1.1.1.49) activity were carried out to correlate the morphological and functional changes in the adrenals of rats treated with both dexamethasone phosphate and FAD.
MATERIALS AND METHODS

Wistar strain male rats weighing about 150 g were used. Dexamethasone phosphate (0.25 mg/animal/day; Nippon Merck Banyu Co.) was administered intraperitonealy once a day for 5 days. FAD (10 mg/animal/day; Toa Eiyo Co.) was also administered in a similar manner to rats simultaneously with dexamethasone phosphate. The rats were killed 6 hr after final administration of steroid hormone and FAD. After decapitation, the bilateral adrenals were removed and were fixed in glutaraldehyde solution, immersed in OsO₄ solution and then embedded in Epon 812 for electron microscopy. The thin epoxy sections were doubly stained with uranyl acetate and lead citrate. G-6-PD activity in the adrenals was determined as follows.

The adrenals were placed in a glass tissue grinder with 4 ml of chilled 0.14 M sodium chloride. After homogenization, the homogenate was carefully poured into a Potter Elvehjem teflon pestle homogenizer and rehomogenized. This homogenate was centrifuged at about 20,000 × g for 30 min at 4°C, and an enzyme assay was carried out on the supernatant. Adrenal activity of G-6-PD was determined by the method of ZINKHAM (2). Assays were performed in cuvettes with a light path of 1.0 cm. Each cuvette contained 0.2 ml of adrenal supernatant, 1 ml of 0.19 M Tris-HCl buffer (pH 7.8), 0.1 ml of 0.03 M MgCl₂, 0.2 ml of 0.002 M nicotinamide adenine dinucleotide phosphate (NADP), 0.1 ml of 0.02 M 6-phospho-gluconate and water to a final volume of 3.0 ml. 0.1 ml of 0.02 M glucose-6-phosphate, was added to a second cuvette. The reaction was started by the addition of NADP, and the change of optical density was recorded for a period of 10 min in a double beam spectrophotometer (Hitachi 124 Spectrophotometer). The activity value of G-6-PD was obtained by subtracting the rate observed in the first cuvette from that of the second. Enzyme values were expressed as the changes in optical density per minute per gram of soluble protein. The concentration of protein in the adrenal supernatant was determined by the method of LOWRY et al. (3). Egg albumin was used as a standard.

RESULTS

Electron microscopic findings of the fascicular cells in the adrenals.

Since a remarkable atrophy had been found previously in the fascicular zone of the adrenal gland of rats after administration of dexamethasone phosphate alone (1), comparative study of the fascicular cells from experimental and intact animals was carried out.

In the fascicular cell of intact animals gloubular or oval shaped nuclei were found to be located in the center of the cells. Mitochondria were distributed densely in the cytoplasm. The cristae mitochondrials were small vesicular or tubular in shape. Smooth-surfaced endoplasmic reticulum and free ribosomes were also observed (Fig. 1).
After administration of dexamethasone phosphate alone, the fascicular cells and their nuclei were irregular in shape. Characteristically, in many of the cells the nuclei were eccentrically located so as to be almost contiguous to the cell membrane. A marked reduction in the development of mitochondria, smooth-surfaced endoplasmic reticulum and free ribosomes were obvious in contrast to intact fascicular cells. By contrast, lipid droplets and microbodies were distri-
Fig. 2. Fine structure of adrenal fascicular cells of a rat treated with dexamethasone phosphate (0.25 mg/animal/day) for 5 successive days. ×10,000

Buted more densely than in intact cells (Fig. 2).

In the case of simultaneous administration of FAD and dexamethasone phosphate, although the cell nuclei were shown to be somewhat more irregular compared to normal cells, they were located near the center of cells as in normal intact cells. Mitochondria and free ribosomes were distributed as densely as in normal cells, whereas the smooth-surfaced endoplasmic reticulum was markedly decreased.
in development just as in the dexamethasone-treated rats. Furthermore, the number of small lipid droplets per unit cytoplasmic area was about one-half of that in animals treated by dexamethasone alone. The number of microbodies per unit cytoplasmic area, on the other hand, was about one-half in comparison with intact cells (Fig. 3).
Table 1. Counts of profiles of organellae per 50 \( \mu \text{m}^2 \) of cytoplasmic area of adrenal fascicular cells of the rat. Figures represent the average count for 20 cells each from the untreated animal, dexamethasone phosphate and flavin adenine dinucleotide-treated, and dexamethasone phosphate-treated rats.

<table>
<thead>
<tr>
<th>Organellae Type</th>
<th>Normal</th>
<th>Dexamethasone phosphate (0.25 mg/animal/day) + FAD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitochondria</td>
<td>30±5(^a)</td>
<td>28±9</td>
</tr>
<tr>
<td>Smooth surfaced endoplasmic reticulum</td>
<td>146±46</td>
<td>106±52</td>
</tr>
<tr>
<td>Lipid droplets</td>
<td>3±1</td>
<td>36±11</td>
</tr>
</tbody>
</table>

\(^a\) Standard deviation

Table 2. Effects of dexamethasone phosphate and flavin adenine dinucleotide on the adrenal glucose-6-phosphate dehydrogenase activity of the rat. Determination of this enzyme activity was carried out according to modified method of ZINKHAM (2).

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Number of cases</th>
<th>G-6-PD activity (( \Delta \text{A}430 \text{ nm O.D.}/\text{min/g protein} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4</td>
<td>139.0±5.7(^a)</td>
</tr>
<tr>
<td>Dexamethasone phosphate</td>
<td>3</td>
<td>43.2±6.7 ( p &lt; 0.01 )^(^b)</td>
</tr>
<tr>
<td>Dexamethasone phosphate + FAD</td>
<td>4</td>
<td>93.0±7.8 ( p &lt; 0.01 )</td>
</tr>
</tbody>
</table>

\(^a\) Standard deviation
\(^b\) In \( t \)-test

Numerical assessments of the number of the organellae in adrenal fascicular cells of rat treated with dexamethasone phosphate and simultaneously with FAD were carried out as shown in Table 1.

Adrenal G-6-PD activity was found to have decreased remarkably after administration of dexamethasone phosphate alone. However, it was evident that the decrease of this enzyme activity was prevented by FAD administered simultaneously with dexamethasone phosphate (Table 2).

**DISCUSSION**

It was thought worthwhile to determine the adrenal G-6-PD activity in the rats treated simultaneously with dexamethasone phosphate and FAD in order to clarify one of the preventive mechanisms of FAD on the induction of adrenal atrophy by dexamethasone phosphate because a high G-6-PD activity exists in the adrenal cortex and this enzyme activity must be closely related to the steroidogenesis in adrenals (4).

In the present study, the adrenal G-6-PD activity was reduced by intraperitoneal administration of dexamethasone phosphate. However, the reduction of the enzyme activity was fairly well prevented by FAD administration. This suggests that the preventive effects of FAD administration on the induction of
adrenal atrophy by dexamethasone phosphate are closely related to the activation of G-6-PD activity in the adrenals.

Electron microscopic observations on the adrenals treated with both dexamethasone phosphate and FAD suggest that the function of the adrenals of rats is depressed with dexamethasone phosphate administration alone, whereas adrenal function remains nearly normal after administration with FAD plus dexamethasone. This was particularly clear from the changes in number of cell organelles, especially lipid droplets, and from the observations of the location of the nucleus in the fascicular cell of the adrenals.

It still remains obscure whether or not ACTH participates in the preventive effects of FAD when adrenal atrophy was induced by dexamethasone phosphate.

It is well known that the secretion of ACTH from the pituitary gland is depressed by dexamethasone administration (5) and that ACTH stimulates the steroidogenesis in the adrenals through activation of G-6-PD activity (4). Therefore, we have attempted to determine the effects of FAD on the G-6-PD activity in the hypophysectomized rats in order to know the relationships between FAD and ACTH on the G-6-PD activity (6). The most interesting fact was that the decrease of this enzyme activity in the hypophysectomized rat was well prevented by FAD administration, suggesting that FAD activates the G-6-PD in the adrenal cortex even if the secretion of ACTH was depressed or failed.

Furthermore, in the presence of ACTH, the effect of FAD was to potentiate the actions of ACTH to increase the weight of, and G-6-PD activity in adrenals of hypophysectomized rat.

These findings suggest that FAD probably acts against induction of the adrenal atrophy by means of 1) preservation of G-6-PD activity and 2) a trophic action to the adrenal gland itself in cooperation with ACTH.

We wish to express our thanks to Prof. A. Yamauchi, Department of 2nd Anatomy, Iwate Medical University, for his valuable advice and criticism.

REFERENCES