Comparison of the Effects of Adrenocorticotrophic Hormone on the Steroidogenic Activity and Ultrastructure of Adrenal Cortex*

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SUMMARY

Comparison of ultrastructural and biochemical response to adrenocorticotropic hormone (ACTH) in superfused adrenals or cell suspension and in adrenals of hypophysectomized rats showed that (a) steroidogenic response can take place in superfused glands that have suffered gross structural damage and contained no detectable intact mitochondria; (b) steroidogenesis of cell suspensions takes place without dilation of the endoplasmic reticulum; (c) adrenals of 1-day posthypophysectomized rats respond normally to ACTH in terms of ultrastructural changes even though they have lost their responsiveness to ACTH in terms of steroidogenesis; (d) these ACTH-induced ultrastructural changes in adrenals of hypophysectomized rats are blocked by the prior administration of cycloheximide and postulated to be dependent upon the translation of preformed mRNA with a longer half-life than the mRNA coding for the labile steroidogenic protein.

The mammalian tissues concerned with the biosynthesis of steroid hormones contain mitochondria that are unique both in their ultrastructure and in the presence of a separate electron chain for various steroid oxygenases. These unique features have led to extensive ultrastructural studies and attempts to correlate mitochondrial morphology and steroidogenesis. Unfortunately, the ultrastructural studies and biochemical studies were usually carried out in different laboratories, resulting in much uncertainty on the interpretation of the ultrastructural changes. We wish to report here that (a) ACTH can induce the characteristic ultrastructural changes without significant stimulation of steroidogenesis; and (b) in vitro ACTH-induced steroidogenesis can proceed in the absence of normal cellular structure and certain structural changes normally associated with hormone stimulation.

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The abbreviation used is: ACTH, adrenocorticotropic hormone.

EXPERIMENTAL PROCEDURE

Normal and hypophysectomized female Sprague-Dawley rats weighing approximately 150 g were purchased from the Hormone Assay Laboratories, Chicago. The animals were maintained on a high protein diet with water ad libitum. The adrenals were excised rapidly, immersed into a solution of 3.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3, and cut into small pieces approximately 1 mm³. For orientation purposes, each small block was cut so that it contained a reference point a piece of the capsule. After 3 hours of aldehyde fixation, tissues were post-fixed in 1% osmium tetroxide-0.1 M phosphate buffer (pH 7.3). Dehydration was accomplished in a graded series of ethanol. Tissue blocks were then embedded in Epon 812 and polymerized stepwise at 37°, 45°, and 60° over the next 5 days. Thick sections stained with methylene blue were examined by light microscopy. The zona fasciculata was identified and trimmed for thin sectioning. Thin sections were stained in uranyl acetate followed by lead citrate and photographed in an RCA EMU 3H electron microscope.

In the case of the superfused glands (see accompanying paper), the tissues were fixed and examined in the same manner with the additional precaution that samples from different depth were examined. This precaution was taken to avoid the possibility that any abnormality may result from anoxia of the inner portion of the capsule and is not representation of the over-all structure. Cell suspensions were prepared according to Sayers et al. (1). Although the total time for their preparation lasted several hours, their response to ACTH by increasing steroid output (1) was readily confirmed.

RESULTS

Work from several laboratories has established the fact that in the first day after hypophysectomy the adrenal cortex undergoes relatively little ultrastructural changes while losing most of its responsiveness to ACTH in terms of steroid output (2, 3). We therefore, examined the acute ultrastructural changes inducible by ACTH in 1-day posthypophysectomized rats. The results are shown in Figs. 1 to 3.

Two of the characteristic normal responses to ACTH, namely, dilation of the endoplasmic reticulum and penetration (4–8) of mitochondria into lipid droplets to form "myelin-like" structures (9), were still observed in the hypophysectomized animals (Figs.
FIGS. 1–4.
Legend on page 6681
types of cells are observed. One type consists of obviously
droplets into lipid droplets. In spite of such drastic structural
diminution (Fig. 7). There also appears to be coalescence of the lipid
an extent that one can no longer see an individual mitochow
outer membranes of the mitochondria have disintegrated to such
branes, were seen (Fig. 6). In the vast majority of samples, the
results of Tnit et al. (3) that these glands retained one-half of
the diminution of the mitochondrial cristae, and a general
fuzziness of structure. Glands superfused without ACTH for 6
hours underwent drastic ultrastructural change. No intact
mitochondrion was seen in the electron micrographs. In very rare
instances, clearly defined mitochondria, with broken outer
membranes, were seen (Fig. 6). In the vast majority of samples, the
outer membranes of the mitochondria have disintegrated to such
an extent that one can no longer see an individual mitochondrion
(Fig. 7). There also appears to be coalescence of the lipid
droplets into larger droplets. In spite of such drastic structural
changes, analysis of ACTH-stimulated steroid output confirmed
the results of Tait et al. (3) that these glands retained one-half of
their steroidogenic ability. Glands superfused for 3 hours showed
structures that are in between those seen in control glands and in 6 hour superfused glands.

These degenerative changes observed in superfused glands are
most likely due to anoxia. When cell suspensions were examined
(after 6 hours have elapsed from the sacrifice of the animal), two
types of cells are observed. One type consists of obviously
damaged cells with grossly altered structures. The other type
consists of apparently intact cells with all the usual characteristic
structures of adrenal cortex cells. When inebuated with ACTH
to stimulate steroid synthesis, these cells also contain the myelin
structure formed by penetration of mitochondria into lipid droplets.
However, the dilation of the endoplasmic reticulum seen
in vivo was absent (Fig. 8).

FIG. 1 (upper left). ACTH-induced dilation of endoplasmic reticulum. The electron micrograph shows a portion of an adrenal cortex cell after ACTH treatment of 1-day hypophysectomized rats. One unit of ACTH was given intravenously and the rats were killed 30 min later. The adrenals were fixed as described under "Materials and Methods" in similar manner as other workers (7, 8). Note the dilation of the endoplasmic reticulum as vesicles in the cytoplasm. Mitochondria are round with vesicular cristae. The lipid droplets are in the form of amorphous bodies of variable electron density. Magnification approximately \( \times 5,300 \).

FIG. 2 (upper right). ACTH-induced fusion between a mitochondrion and a lipid droplet. The sample for electron microscopy was the same as in Fig. 1. A mitochondrion is shown here penetrating into lipid droplets to form a myelin-like structure. Magnification approximately \( \times 20,000 \).

FIG. 3. (lower left). The effect of cycloheximide. The samples
were prepared as in Fig. 1 with the exception that each rat received
10 mg of cycloheximide (intraperitoneal injection) 10 min before
ACTH injection. The cycloheximide treatment abolished the effects of ACTH seen in Figs. 1 and 2. Magnification approximately \( \times 11,000 \).

FIG. 4 (lower right). Adrenal gland before superfusion. The adrenals taken from the normal rat were suspended in Krebs-Ringer phosphate bicarbonate buffer. The glands were fixed just before the start of superfusion (see accompanying paper (13) for superfusion technique). The fixation was as described under "Materials and Methods." The micrograph shows the normal structures of adrenal cells: (a) the nucleus is round to oval and contains peripheral heterochromatin; (b) an abundant smooth-surfaced endoplasmic reticulum; (c) numerous mitochondria with predominantly vesicular cristae; (d) round amorphous lipid droplets. Magnification \( \times 5,300 \).

Discussion

The interaction between the mitochondria, the endoplasmic reticulum, the cytosol, and the lipid droplets plays an undoubtedly essential role in hormone-induced steroidogenesis. The mechanism of this interaction is, however, still obscure. It is well established that the hormone acts via cyclic adenosine 3',5'-monophosphate which exerts a myriad of biochemical effects: (a) activation of cholesterol esterase (11); (b) synthesis of a labile steroidogenic protein (19); (c) synthesis of mRNA for the labile steroidogenic protein (13); (d) activation of RNA polymerase (14); (e) normal maintenance of the gland (15); (f) regeneration of atrophied gland (16); and (g) cytodiifferentiation of embryonic adrenal cortex cells to "mature" adrenal cortex cells (11). Ultrastructurally, the hormone causes: (a) dilation of the endoplasmic reticulum (4-8); (b) occasional penetration of mitochondria into lipid droplets to form a myelin-like structure (9); (c) extrusion of lipids from the cells (18); (d) normal maintenance of the gland; (e) regeneration of atrophied gland (19); and (f) maturation of fetal adrenal cortex cells (20). If one were to divide the hormonai effects into two broad categories, tropic and trophic activities, the first two biochemical and the first four ultrastructural effects may be considered as tropic effects directly related to the process of steroidogenesis. Among these, the activation of cholesterol esterase has a clear physiological function, namely, the conversion of stored cholesterol ester to cholesterol for the side chain demolase complex. The transport of the generated cholesterol to the mitochondria is not yet understood. Several hypotheses have been proposed, including the fusion of mitochondria with lipid droplets, the participation of an intracellular cholesterol binding protein for transport (21), and some undefined function of the labile steroidogenic protein (22). The carrier protein (or proteins) may serve the role of a carrier not only for cholesterol, but also for the intermediates and the final products which must move between the mitochondria and the endoplasmic reticulum and then be secreted. The transport, or movement, of the intermediates and products may be associated with the dilation of the endoplasmic reticulum.

The extrusion of lipid droplets was reported recently, using
tissue fixation by perfusion (18). By this method, it was found that the adrenal cortex contains many "canals" between cells and that, upon ACTH stimulation, lipid droplets were found in these canals. The author postulated that the standard method of tissue fixation causes the collapse of these canals and that this is the reason why the extrusion of lipid droplets was not observed by other workers. However, it is difficult to see how the collapse of the canals would put the lipid droplets back inside the cells and it could be argued that these extracellular lipid droplets are artifacts of the perfusion-fixation technique.
It is clear from the above discussion that many interesting ultrastructural changes take place in ACTH-stimulated adrenal cortex and many intriguing hypotheses have been proposed to implicate these changes in the process of steroidogenesis. The work described in this paper was undertaken to attempt to correlate these structural changes with biochemical events. The results are largely negative. The normal structural changes in response to in vitro ACTH administration were observed in 1-day posthypophysectomized rats which had lost the ability to respond to ACTH in terms of steroidogenesis. On the other hand, superfused glands which had undergone extensive structural deterioration and contained no intact mitochondria can still respond very well to ACTH in terms of steroidogenesis. This lack of correlation suggests that these events may be independent of each other as discussed below.

The steroidogenic activity of ACTH has been shown to be dependent upon the translation of a preformed mRNA to form a "labile steroidogenic protein." This mRNA has been estimated to have a half-life of approximately 6 hours and would have largely decayed in 1-day posthypophysectomized rats, so that the glands can no longer respond significantly to ACTH in the medium (13). However, ACTH still induces the characteristic normal ultrastructural changes and the addition of cycloheximide blocks these changes. It is possible that these ultrastructural changes are also dependent upon translation of a preformed mRNA which has a longer half-life than the mRNA for the labile steroidogenic protein. However, these changes are clearly not sufficient to cause increased steroidogenesis.

Cell suspensions have been shown by several laboratories (1) to respond to low physiological concentrations of ACTH. Electromicroscopic examination of such preparations show that they contain a mixture of intact and broken cells. Although the numbers of intact cells cannot be determined accurately, it is estimated that at least one-third of the cells are intact and possessing normal structures. The results of our assay show that these intact cells are responsive to a minimum dose of 10 μunits of ACTH. These cells, however, do not respond to ACTH with all the normal ultrastructural changes. Fusion of mitochondria with lipid droplets is still seen, but the dilation of endoplasmic reticulum was absent for unknown reasons. Thus, it may be concluded that steroidogenesis can take place without concomitant dilation of the endoplasmic reticulum.

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