MORPHOLOGICAL DIFFERENTIATION
OF ADRENAL CORTICAL CELLS INDUCED BY A
40-MINUTE EXPOSURE TO CORTICOTROPHIN

A. JOSEPHINE MILNER*

Department of Pharmacology, Medical School,
Hills Road, Cambridge, CB2 2QD, England

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The effects of adrenocorticotrophin (ACTH) on adrenal cortical cells fall into two
categories: (i) the trophic effect, and (ii) the acute steroidogenic effect. Recently
ACTH has been shown to induce the functional differentiation of cortical cells,
a process which involves nucleic acid and protein synthesis and which requires 4 days
for completion (Kahri, 1970; Milner, 1971, 1972). This differentiation of cortical cells
in response to ACTH may be classified as a trophic effect of the hormone and evidence
from tissue culture studies indicates that it precedes the ability of the cells to show
an acute steroidogenic response to ACTH (Milner, 1971). The process of differentiation
includes morphological changes in those organelles which are the sites of steroid
synthetic activity, namely the smooth endoplasmic reticulum and the mitochondria
(Kahri, 1966). The morphological transformation is accompanied by an increase in
the steroidogenic capacity of the cortical cells (Milner & Villee, 1970). Much of the
above information has been obtained using a tissue culture system in which the
functional differentiation of cortical cells grown from foetal rat adrenals was induced
by the daily addition of ACTH to the culture medium. The present investigation was
designed to determine whether regular exposure to ACTH over the differentiation
period is in fact necessary, or whether a single exposure to ACTH is sufficient to
toggle a process of differentiation requiring 4 days for completion.

Tissue cultures were grown from foetal rat adrenals as described previously
(Milner, 1972). On the 9th day of culture ACTH (1 i.u./ml medium) was added to the
experimental cultures. After 40 min the medium was replaced with fresh medium
without ACTH. The cells were maintained in culture for a further 4 days before they
were prepared for electron microscopy. Control cultures were subjected to the same
treatment, omitting the addition of ACTH. The cells were fixed in situ in 5% glutaraldehyde at pH 7.4, post-fixed in osmium tetroxide and embedded in Taab
resin (Milner, 1972). Ultrathin sections were stained in lead citrate for 3 min and
examined in a Phillips E.M. 300 electron microscope.

Fully differentiated cells in the rat adrenal cortex characteristically contain a well-
developed smooth endoplasmic reticulum and abundant mitochondria. In the rat the
mitochondria are distinctive in that they contain vesicular cristae formed from the
inner membrane. When cultured in the absence of ACTH for 13 days the cortical cells
were small and the cytoplasm contained a poorly developed smooth endoplasmic

* Beit Memorial Research Fellow.
reticulum and mitochondria with vesiculo-lamellate cristae (Plate, fig. 1). In contrast those cells which had been exposed to ACTH for 40 min on culture day 9 and subsequently maintained in culture for a further 4 days were morphologically similar to cortical cells of the foetal rat adrenal in vivo. In particular they contained a well developed smooth endoplasmic reticulum and enlarged mitochondria with vesicular cristae (Plate, fig. 2).

The results demonstrate that a single 40-min exposure to ACTH is sufficient to induce the morphological differentiation of adrenal cortical cells. The differentiation process requires 4 days for completion and investigations to establish the sequence of events after exposure to ACTH are in progress.

REFERENCES

DESCRIPTION OF PLATE

Figs 1 and 2 are electron micrographs at the same magnification. (× 31 500.)

Fig. 1. Rat adrenal cortical cells cultured in the absence of adrenocorticotrophin (ACTH) for 13 days. A high magnification to show the mitochondria (M) with vesiculo-lamellate cristae which are characteristic of cortical cells cultured in the absence of ACTH. Such mitochondria were not observed in any of the other cell types present in the tissue cultures and were used as markers for identifying the cortical cells. Note, in addition, the poorly developed smooth endoplasmic reticulum, the diffuse scattering of glycogen particles, which are more electron-dense than the ribosomes, and the lipid droplets (L). The close association between two cells is also visible (arrow).

Fig. 2. Rat adrenal cortical cells after a short-term exposure to ACTH. Part of a cell which was exposed to ACTH for 40 min on the 9th day of culture and then fixed for electron microscopy 4 days later. ACTH has induced the formation of vesicular cristae in the mitochondria (M) which are also enlarged relative to those observed in untreated cells (cf. fig. 1). In addition, the smooth endoplasmic reticulum is well developed and is often closely associated with the mitochondria (arrow). The lipid droplets (L) are larger and less electron-dense than those in the untreated cells.