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# Ultrastructure and Morphometry of ACTH-Producing Cell in the Rat Anterior Pituitary Gland Stimulated by Lysin-Vasopressin and Prostaglandin E1

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**Summary.** The aim of this study was to investigate the qualitative and quantitative changes of ACTH-cells in the rat after application of a specific and a non-specific stimulus. A CRF-analog (lysin-vasopressin) and a prostaglandin (prostaglandin E1) were used. 40 rats were injected lysin-vasopressin or prostaglandin E1, respectively, for 4 weeks. The pituitary glands were investigated by means of light microscopy, electron microscopy and morphometry. Activation of the ACTH-cells could be observed after use of both substances, the effect of lysin-vasopressin being more intense than that of prostaglandin E1. Enlargement of the nucleus, the cytoplasm and the organelles involved in hormone-production and -transport were found and verified by morphometry. Additionally an increase in number of the cells could be demonstrated. Prostaglandin influenced not only ACTH-cells, but also other cells of the anterior pituitary.

**Key words:** Pitutary – Ultrastructure – Vasopressin – Prostaglandin – Morphometry.

**Zusammenfassung.** Ziel dieser Untersuchung war es, quantitative and qualitative Änderungen der ACTH-Zelle in der Rattenhypophyse nach Gabe eines spezifischen und eines unspezifischen Stimulus zu untersuchen. Es wurden ein CRF-Analogon (Lysin-Vasopressin) und ein Prostaglandin (Prostaglandin E1) benutzt. Jeweils 20 Ratten wurden für 4 Wochen Lysin-Vasopressin oder Prostaglandin E1 intraperitoneal injiziert. Die Hypophysen wurden licht- und elektronenoptisch sowie morphometrisch untersucht. Nach Gabe beider Stoffe konnte man eine Aktivierung der ACTH-Zellen beobachten. Die Vergrößerung des Kerns, des Cytoplasmas und der an der Hormonherstellung beteiligten Zellorganellen ließ sich morphometrisch nachweisen, wobei die Wirkung von Lysin-Vasopressin ausgeprägter war als die des Prostaglandins. Darüber hinaus konnte eine Zunahme der ACTH-Zellzahl gefunden

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und mit einem statistischen Test als signifikant nachgewiesen werden. Im Gegensatz zu Lysin-Vasopressin erstreckte sich die Wirkung des Prostaglandins auch auf andere Hypophysenzellen.

## I. Introduction

Corticotropin-releasing factor (CRF) is a well-known stimulus for the anterior pituitary gland (Schally et al., 1968; Danowski, 1976; Hiroshige et al., 1977), and its chemical structure is similar to the oligopeptides of the posterior pituitary gland (Schally et al., 1968). Among these peptides it is lysin-vasopressin which is most used in the clinical field, because of its CRF-like effect. After injection of this substance corticosteroids rise in the blood as a result of ACTH-stimulation (Bethge et al., 1967; Brostoff et al., 1968; Czarny et al., 1968; Labhart et al., 1969; Feurle et al., 1970), an effect verified by investigations on rats in vivo (De Wied, 1961) and in vitro (Portanova and Sayers, 1973). Apart from this specific method of stimulation of a special part of the anterior pituitary gland, there exist non-specific methods, using different biochemical pathways of cell-activation. Among the non-specific methods it is the use of prostaglandins which has recently attracted the special interest of biochemists. The rise in corticosteroid levels after the administration of prostaglandins is generally interpreted to be the result of a rise in ACTH (Peng et al., 1970; Vale et al., 1971; Hedge and Hanson, 1972; Hedge, 1976, 1977). Prostaglandins also stimulate the production of various other hormones of the pituitary gland (McLeod and Lehmeyer, 1970; Schofield, 1970; Hertelendy, 1971; Burton et al., 1975; Kraicer, 1975; Drouin and Labrie, 1976; Hedge, 1977), acting directly and indirectly – via the hypothalamus – on the pituitary gland (Hedge, 1977).

The effects of lysin-vasopressin and prostaglandins have been studied extensively in biochemical investigations which often use cell cultures or fractions of cells (Kraicer, 1975; Hedge, 1977). In living creatures, all biochemical pathways are bound to structures which can be examined by morphological means. It would therefore be interesting to see whether or not a change in the appearance of the ACTH-cell could be observed under the influence of these substances. At the light microscopic level extensive studies on the pituitary gland under influence of corticosteroids have been carried out by Stein (1955) who observed the morphological response to suppression of this gland. Preliminary work (Saeger and Caselitz, 1974) showed that a long term stimulation of ACTH-cells causes marked hyperplasia. The aim of the following studies was to examine the change in the number and structure of ACTH-cells after administration of a CRF-analog (lysin-vasopressin) and a prostaglandin (prostaglandin E1) using electron microscopy and morphometry.

## II. Materials and Methods

44 male Wistar rats, weighing between 200 and 300 g, were held for 4 weeks under standard conditions. They were given Altromin-diet and water ad libitum. All substances were injected intraperitoneally. 20 rats received 0,5 U lysin-vasopressin each day for the first 14 days of the experiment. The dose was doubled for the following 14 days.

10 rats received 10 µg prostaglandin E1 and 10 15 µg of this substance each day for the first 14 days. This dose was doubled in each group for the last 14 days. 4 further rats made up the control group.

The rats were killed by either anaesthesia and perfused with glutaraldehyde. The adrenal glands, heart, lungs, liver, spleen and kidneys were prepared for light microscopy. The pituitary glands were fixed in 3% glutaraldehyde in cacodylate buffer, followed by osmic post fixation and embedded in Epon 812. For preliminary light microscopy semi-thin sections were stained by toluidin-blue. Ultrathin sections, obtained with a Reichert Ultramikrotom, were stained with uranyl acetate and lead citrate and examined with a Zeiss Elmiskop EM 9-S2.

Every ACTH-cell was photographed at a preliminary magnification of 3000. For morphometric studies a final magnification of 9100 was obtained and morphometry was performed using a square grid of $2 \times 2$ mm, the cross points of which were used as markers for point-counting. The points were counted in the following groups: nucleus, Golgi apparatus, smooth and rough endoplasmic reticulum, granules, mitochondria, other organelles and the so-called undifferentiated cytoplasm (matrix).

The significance of the differences between the components covered by the points was calculated by the student-*t*-test or the Wilcoxon-test, when the former could not be used on statistical grounds (Documenta Geigy, 1975). Assuming random sampling of the photographs the statistical significance of the different frequencies of ACTH-cells in the photographs were calculated by the chi$^2$-test[1].

## III. Results

### *Control Group*

The pituitary glands were stained with performic acid-alcian blue–PAS–Orange G (Adams and Swettenham, 1958) and with lead hematoxylin (Solcia, 1969). The ACTH-cells characterized by their triangular shape, their slight alcian blue staining and their positive staining with lead hematoxylin, showed the signs of different phases of secretion. Specific activation was not observed. In the non-specific toluidin-blue stain the cells could be readily identified by their typical shape (Fig. 1a).

At electron microscope level ACTH-cells could be recognized by the features which have been pointed out by Saeger (1977). They were not very numerous and showed different degrees of activity. Sometimes they occurred in clusters near capillaries, the cell-shape was often triangular or cusp-like, the nucleus excentrically situated. Endoplasmic reticulum was moderately developed, free ribosomes were numerous, and the Golgi apparatus well established. The mature granules were electron-dense and situated in the vicinity of the cellular membrane. Lysosomes were only rarely found. The mitochondria were generally of oval shape (Fig. 1b).

### *Prostaglandin E1*

Light microscopy of the heart, lung, liver, spleen and kidney showed no pathological changes. The weight of the adrenals had increased ($P < 0.05$) and morphometry of the cortex showed a significant enlargement mainly due to enlargement of the zonae fasciculata and reticularis. Light microscopy of the pituitary gland schowed an enlargement of the cell-bodies of ACTH-cells, which had moderately increased in number. By electron microscopy one could see enlargement and multiplication of these cells (Fig. 2a and b). The nuclei were generally well developed (Fig. 2a), the endoplasmic reticulum seemed to have

[1] We are indebted to Mister R. Geister, Tropeninstitut Hamburg, for his aid in statistical methods

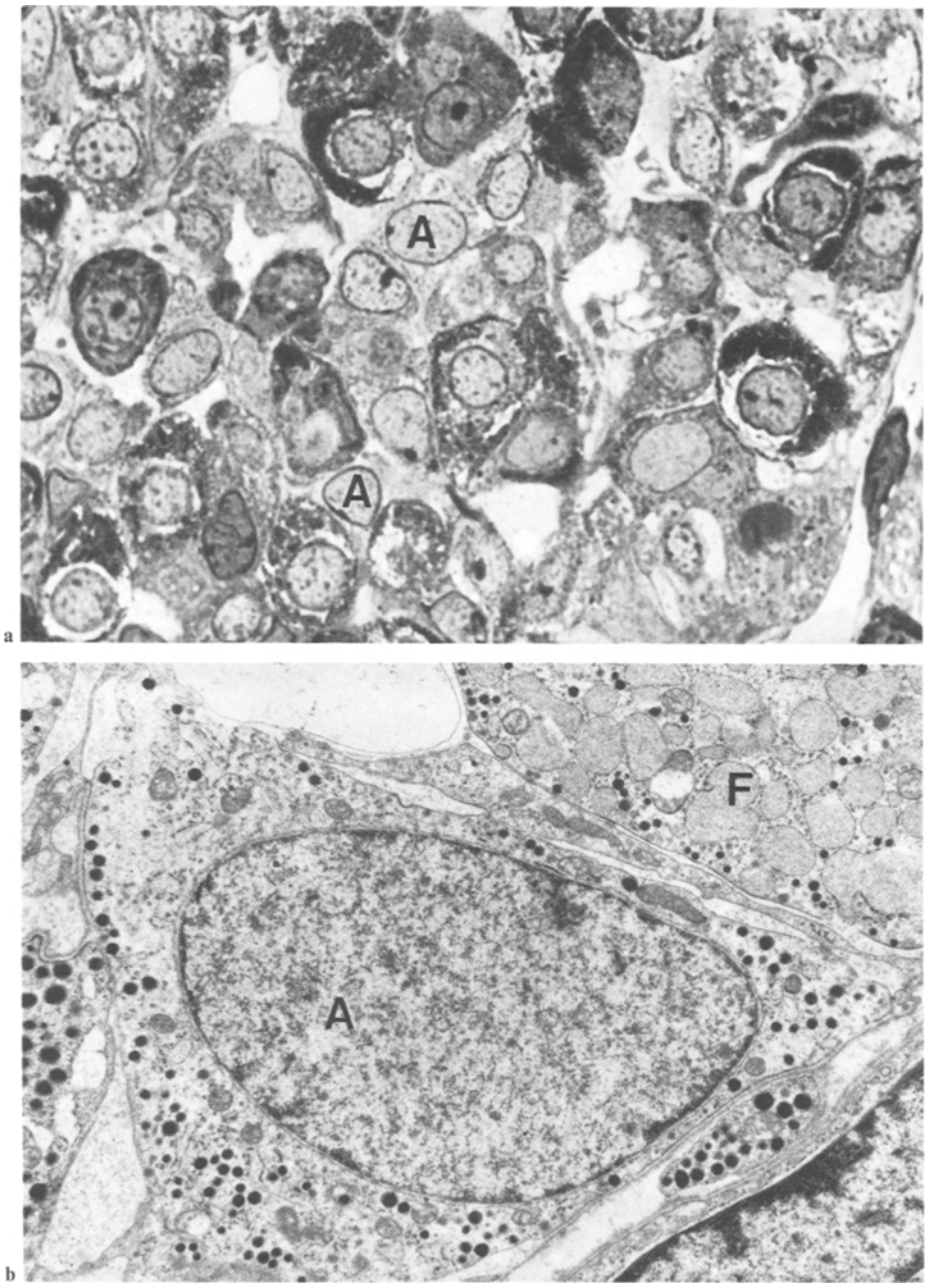


**Fig. 1. a** Normal anterior pituitary gland of a control rat: Some cusp-shaped pale ACTH-cells (*A*) are seen close to capillaries. They show intensely stained secretory granules in the periphery of the cytoplasm. Semithin-section, toluidin-blue, ×1100. **b** Normal ACTH-cell of a control rat: Triangular shape of the ACTH-cell (*A*), round nucleus, sparse endoplasmic reticulum, granules with a diameter from 150 to 240 μm, some round electron-dense mitochondria. Adjacent a FSH-cell (*F*). ×6440

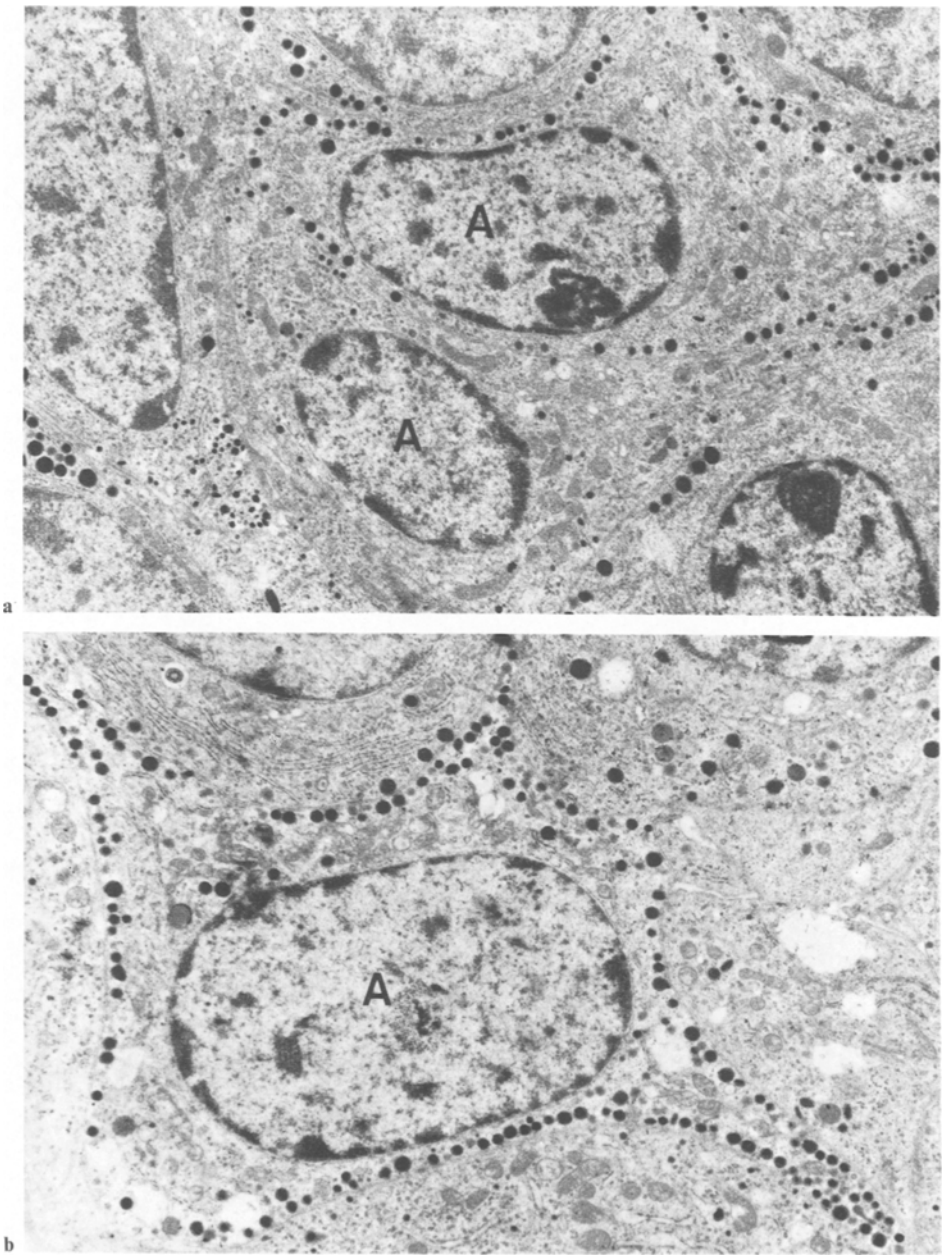


**Fig. 2. a** ACTH-cells after prostaglandin E1: 2 adjacent ACTH-cells (*A*) with typical triangular shape, moderately increased endoplasmic reticulum, many free ribosomes, granules of a diameter from 160 to 190 μm and many oval mitochondria. × 5940. **b** ACTH-cell after prostaglandin E1: Stellate ACTH-cell (*A*) with cytoplasmic branches, endoplasmic reticulum, typically situated granules, few lysosomes and several oval mitochondria. × 5460

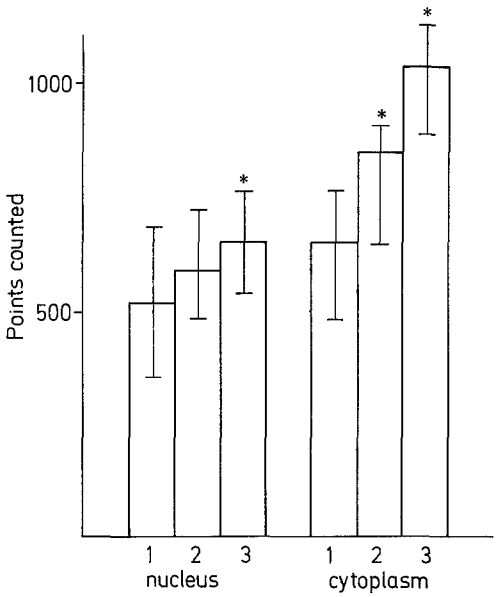


**Fig. 3.** Fraction of nucleus and cytoplasm expressed in counted points. *1* control, *2* prostaglandin, *3* lysin-vasopressin. The amounts of the points are expressed as mean values (with exception of the group 2 of the cytoplasm which shows the median value) and the 95% confidence limits. * Significance $P < 0.05$

changed only slightly whereas the Golgi apparatus had clearly increased in size (Fig. 2a). Sometimes several Golgi fields could be observed in one cell. The mature granules were generally 150 to 250 μm in diameter, and occasionally haloed. They could often be found near the cellular membrane (Fig. 2a and b). Rarely lysosomes (Fig. 2b) and microtubules were observed. The mitochondria were of oval shape and electron-dense (Fig. 2a). Statistical analysis confirmed the qualitative morphological findings. (Whenever the term "significant" is used subsequently a significance of $P < 0.05$ is designated.) The enlargement of the cytoplasm (Fig. 3) and the relative increase in the fraction of the Golgi apparatus were significantly altered from normal. The ratio nucleus/cytoplasm had not changed, and the other organelles showed no significant difference. The analysis of the number of ACTH-cells in one photograph in comparison to the control showed that the increase in number was significant ($chi^2$-test). Not only the ACTH-cells but also the other cells of the pituitary seemed to be stimulated by prostaglandins. In particular the gonadotrophin producing cells showed an increase of endoplasmic reticulum and the Golgi field.

*Lysin-Vasopressin*

The light microscopy of the heart, lung, liver, spleen and kidney showed no pathological changes. The weight of the adrenal glands had increased significantly, as had the diameter of the cortex. The zonae fasciculata and reticularis were enlarged, and a decrease in lipid content was observed.

Light microscopy of the pituitary gland showed a clear enlargement and multiplication of the ACTH-cells. This observation was more striking than that seen in the group of the prostaglandin-treated rats. Because of their well-developed characteristics the ACTH-cells were easily identified in the semi-thin sections stained with toluidin-blue (Fig. 4a).

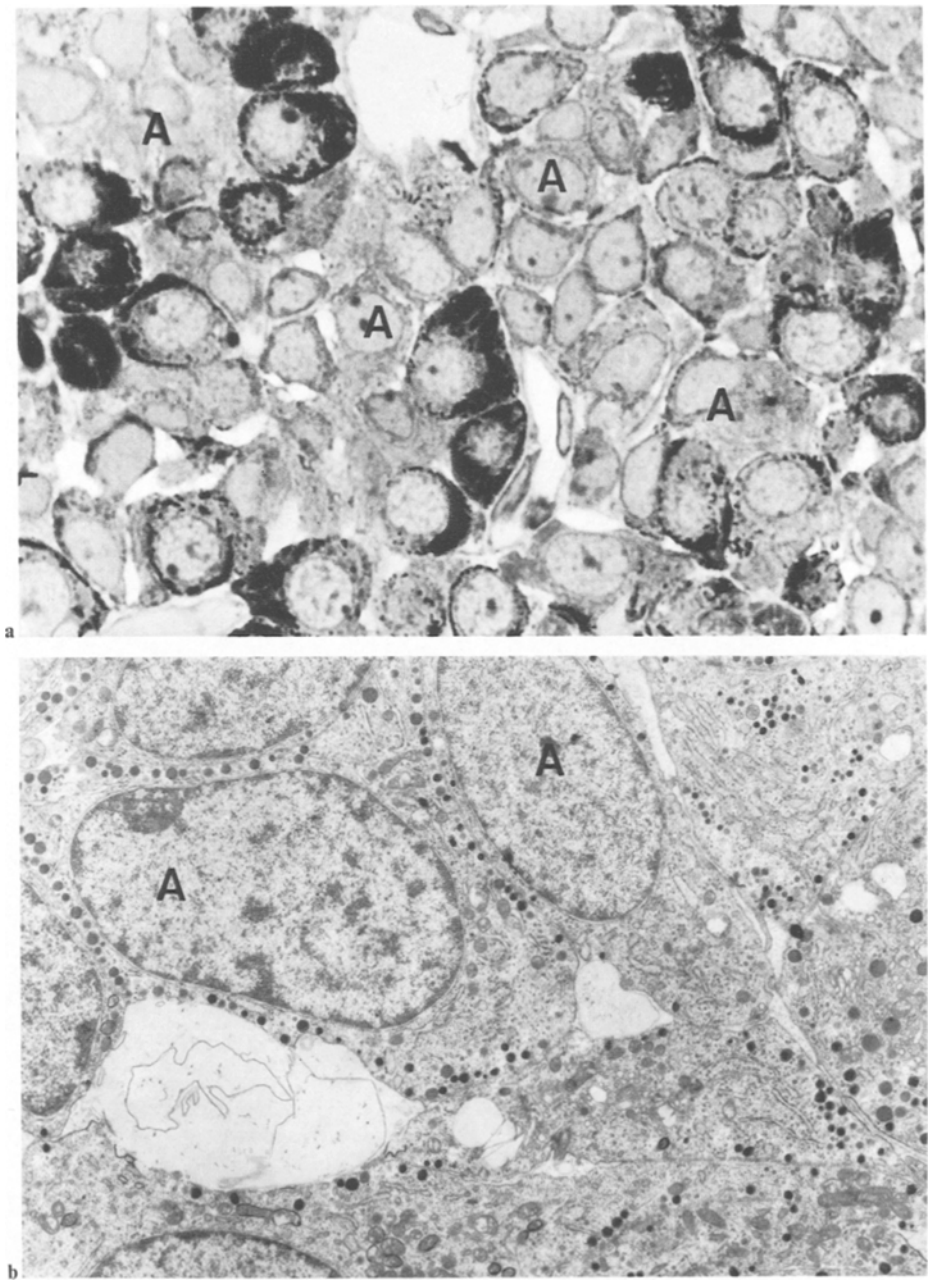


**Fig. 4. a** Hyperplasia of ACTH-cells (*A*) after lysin-vasopressin: Many moderately enlarged ACTH-cells with granules at the periphery, enlarged nuclei and distinct nucleoli. Semithin-section, toluidin-blue, ×1100. **b** ACTH-cells (*A*) after lysin-vasopressin: Cusp-like shape, endoplasmic reticulum in scattered membranes forming parallel layers, well-developed Golgi apparatus with many immature granules, mature granules of a diameter from 140 to 230 µm near the membranes. ×5490

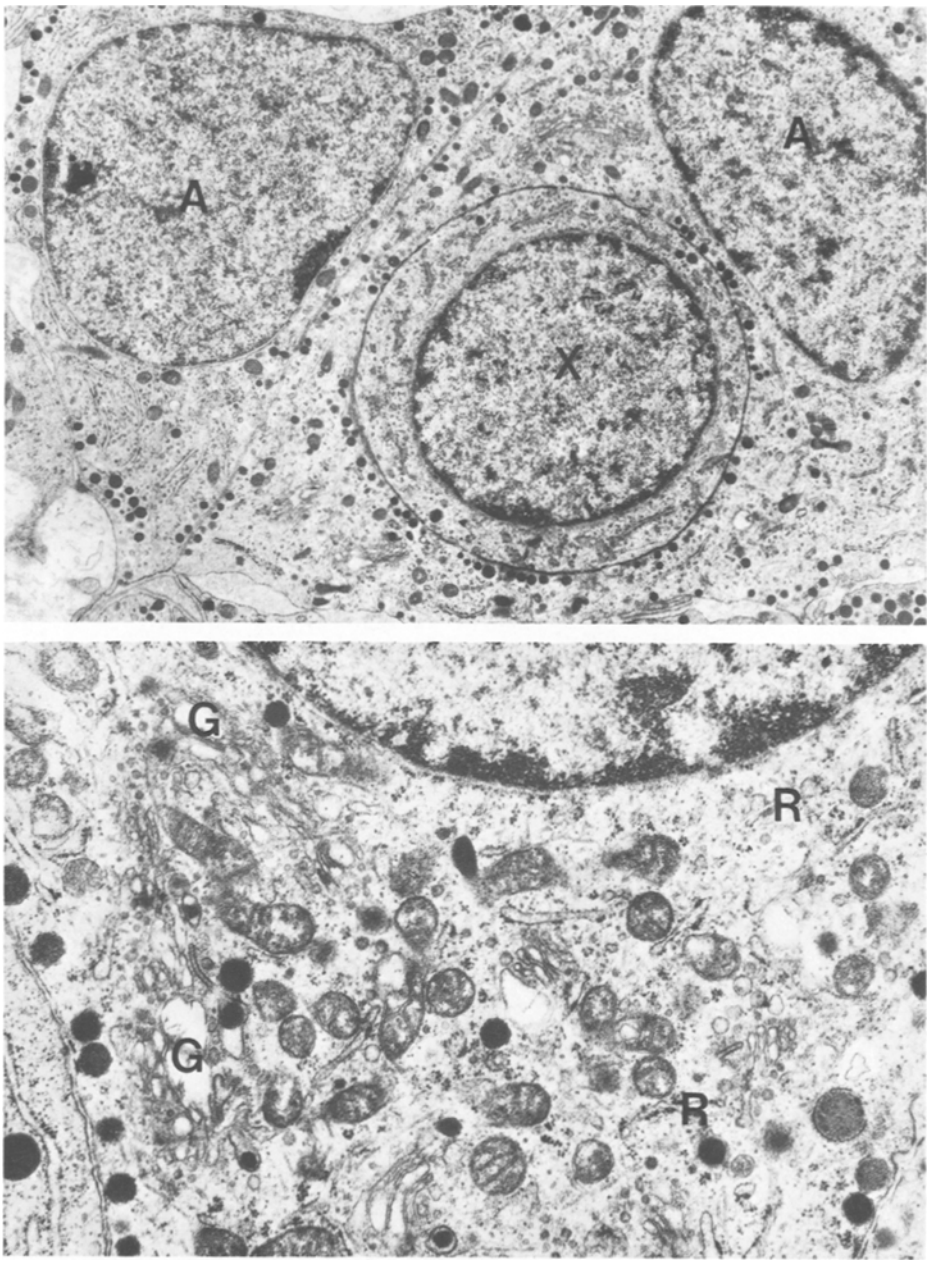


**Fig. 5. a** ACTH-cells (*A*) after lysin-vasopressin: One cell engulfing another (*X*). Well developed Golgi apparatus, granules of a diameter from 130 to 220 µm adjacent to the membrane. ×4200. **b** ACTH-cell after lysin-vasopressin: Enlarged rough endoplasmic reticulum (*R*), many free ribosomes, well developed Golgi apparatus (*G*), many immature granules, mature granules mainly at the membrane. ×12,850

Electron microscopy revealed a distinct enlargement of single ACTH-cells (Figs. 4b, 5a, 5b) which often showed the criteria of the "stellate cell" with so-called "ring figures" (Fig. 5a). The nucleoli of the excentrically situated nuclei were well developed (Figs. 3b, 4a). The endoplasmic reticulum was relatively poor in ribosomes and was present in the form of schattered membranes (Fig. 5a and b). The numerous ribosomes were sometimes disposed in "polysomes" (Fig. 5b). The Golgi apparatus was strikingly well developed, and often several fractions of this organelle could be observed in one cell (Fig. 5b). Nearby, immature granules were seen (Fig. 5b). Mature granules, of a diameter from 150 to 250 μm, sometimes had a halo (Figs. 4b, 5a). Round and generally electron-dense lysosomes were rare (Fig. 5b); there was a slight increase in microtubules. The mitochondria were short, oval and electron-dense (Figs. 4a, 5a and b).

Statistical analysis verified the observations. The enlargement of the nucleus and the increase in cytoplasm were significant (Fig. 3). The relative fractions of the Golgi field and the endoplasmic reticulum were significantly enlarged whereas the fraction of the granules had slightly, but significantly decreased. The ratio of nucleus/cytoplasm had not changed. Analysis of the frequency of the ACTH-cells in photographs showed a significant increase in number ($chi^2$-test).

## IV. Discussion

The main characteristics of the prostaglandins in connection with the pituitary gland have been summarized above and their presence in the gland has been demonstrated by radioimmunoassay (McLeod and Lehmeyer, 1970). Biochemists accept that prostaglandins probably act via the adenylate-cyclase system (Danowski, 1976). Whatever the exact biochemical pathway, the morphology of the pituitary gland shows the clear picture of cell-activation after application of prostaglandin E1. The volume of the whole cell has increased and the organelles involved in hormone production and transport are enlarged. In contrast, lysosomes seem to have decreased in size. A quantitative change seen in the cells after chronic administration was significant, although precise data on the increase are not yet available. It seems likely that ubiquitous existence of the adenylate-cyclase system and the presumed action of prostaglandins on several releasing factors (Hedge, 1977) explain our observation of stimulation of most of the pituitary cells, especially the gonadotrophs. This observation is identical to that of Sato and coworkers (1975) who found an increase in the size of the Golgi apparatus and the endoplasmic reticulum in gonadotrophs after short-term application of prostaglandin E2.

Compared with the "over-all" effect of prostaglandin E1, the effect of lysin-vasopressin appears to be a specific one. The pharmacological characteristics of this CRF-analog have been pointed out in various clinical and experimental investigations (Brostoff et al., 1968; Labhart et al., 1969; De Wied, 1969). In these studies in general, the short-term action of this substance on the hypothalamo-hypophyseal-adrenal axis has been examined, whereas our study aimed

to demonstrate the long-term effects at the morphological level. Our findings are in good agreement with the pharmacological facts. The significant increase in the individual ACTH-cell is followed by an enlargement of the endoplasmic reticulum and the Golgi apparatus, generally interpreted as the sign of increased hormone production. The microtubules seem to have increased slightly in accordance with their function in active hormone secretion (Kraicer, 1975). The granules, however, are diminished in amount in relation to the cytoplasmic volume, an observation to be expected following the chronic stimulation of the cell. This long-term activation also increases the number of cells which have divided. The so-called hyperplasiotropic effect of CRF (Saeger and Caselitz, 1974) is imitated by its analog, and the observed increase in number of ACTH-cells following prostaglandins may be, in turn, due to increased CRF release (Hedge, 1977). Thus, the morphological response of ACTH-cells to chronic administration of prostaglandins and lysin-vasopressin shows the typical features of a stimulated cell-system with qualitative and quantitative differences. The more specific the stimulus the more intense this reaction from the morphological point of view.

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