Ultrastructure of the Corticotrophs from Rats Adrenalectomized Bilaterally

BILATERAL ADRENALEKTOMİ YAPILAN RATLARDA KORTİKOTROP HÜCRELERİN ULTRASTRÜKTÜRÜ

Elvan ÖZBEK*

*Dept. of Histology and Embryology, Medical School of Atatürk University, Erzurum, TURKEY

Summary

It is known that adrenocorticotropic hormone (ACTH) controls adrenocortical steroidogenesis, and the ACTH synthesis is stimulated by a decrease in the plasma level of glucocorticoids. The recent studies show that ACTH is secreted not only from pituitary corticotrophs but also from adrenal gland, and adrenal gland can control itself for glucocorticoid synthesis. So, in the case of absence of adrenal glands, it is expected that pituitary corticotrophs would be stimulated far more to synthesize ACTH, and structural changes in the corticotrophs occur owing to an increase in their functions. In order to examine these possible structural changes, the corticotrophs of the rats killed at 14th day after bilateral adrenalectomy were compared with those of controls at the electron microscopic level. There were small secretory granules arranged in a single row under the plasmalemma in the corticotrophs of control animals. Whereas, in the corticotrophs of adrenalectomized animals, the secretory granules were found to be increased in number and size some of which arranged in 3-4 rows under the plasmalemma, became abundant in the cytoplasm, and accumulated especially in the cytoplasmic processes. In these corticotrophs, granular endoplasmic reticulum (GER) and the Golgi organelle were more developed than in controls; GER was generally arranged in the form of parallel aggregations of cistemae; and the nucleus had a prominent nucleolus. Furthermore, the corticotrophs of adrenalectomized animals had more lysosomes, more cytoplasmic processes extending between neighbor cells, and a bigger area of contact with capillaries than those of controls. In conclusion, it was thought that these findings confirmed synthesis and secretion of ACTH stimulated by bilateral adrenalectomy.

Key Words: Corticotroph, Adrenalectomy, Electron microscopy, Rat

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Özet

Adrenokortikotrop hormon (ACTH)'un adrenokortikal steroidogenezi kontrol ettiği ve plazma glukokortikoid düzeyinin düşmesiyle ACTH sentezinin uyarıldığı bilinir. Son çalışmalar, ACTH'nın sadece hipofizin kortikotrop hücrelerinden değil, adrenal bezden de salgılandığını ve glukokortikoid sentezi için adrenal bezin kendi kendini kontrol edebildiğini göstermektedir. Buna göre adrenal bez yokluğunda, hipofizin kortikotrop hücrelerinin ACTH sentezlemek için şiddetle uyarılması ve artan fonksiyona bağlı olarak bu hücrelerde yapısal değişikliklerin oluşması beklenir. Olası yapısal değişiklikleri araştırmak amacıyla, bilateral adrenalektomi yapıldıktan sonra 14. günde öldürülen sıçanların kortikotrop hücreleri, kontrol grubunun kortikotroplarıyla elektron mikroskobik düzeyde kıyaslandı. Kontrol hayvanlarının kortikotropları, plazmalemma altında tek sıra halinde dizilmiş küçük salgı granüllerine sahipti. Oysa adrenalektomi yapılmış hayvanların kortikotroplarında, salgı granüllerinin sayısının ve büyüklüğünün arttığı, bazen plazmalemma altında 3-4 sıra halinde dizildiği, sitoplazmada yaygınlaştığı ve özellikle sitoplazmik uzantılar içinde biriktiği gözlendi. Bu hücrelerde, granüllü endoplazma retikulumu (GER) ve Golgi organeli, kontrol grubundakilerden daha iyi gelişmişti; GER genellikle birbirine paralel sisterna paketleri şeklinde düzenlenmişti; ve nükleus, belirgin bir nükleolusa sahipti. Ayrıca, adrenalektomi yapılmış hayvanlarm kortikotropları, kontrol grubundakilerden daha fazla lizozoma, komşu hücreler arasına sokulan daha fazla sitoplazmik uzantıya ve kapiller damarlarla daha geniş temas yüzeyine sahipti. Sonuç olarak bulguların, bilateral adrenalektomiyle uyarılan ACTH sentezini ve salgılanmasını yansıttığı düşünüldü.

Anahtar Kelimeler: Kortikotrop, Adrenalektomi, Elektron mikroskop, Sıçan

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Correspondence: Elvan ÖZBEK
Atatürk Üniversitesi
PTT Müdürlüğü, P.K. 1
25171, Erzurum, TURKEY

The pars distalis is the largest subdivision of the hypophysis, and has the typical organization of endocrin tissue. In the pars distalis, using mixtures of acidic and basic dyes, histologists identified three types of glandular cells according to their staining reaction; basophils, acidophils, and chromophobes. One of the basophils is the corticotroph cell (1-3). It is known that the adrenocorticotropic hormone (ACTH) secreted from corticotrophs controls adrenocortical steroidogenesis, and the ACTH synthesis is stimulated by a decrease in the plasma level of glucocorticoids (1,2). However, recent studies show that ACTH is secreted not only from pituitary corticotrophs but also from adrenal gland, and adrenal gland can control itself for glucocorticoid synthesis (4-9). So, in the case of absence of adrenal glands, pituitary corticotrophs are expected to be stimulated far more to synthesize ACTH, and structural changes are expected to be occurred in the corticotrophs owing to an increase in their functions. In this study, it was aimed to examine those possible structural changes. For this reason, the corticotrophs of rats adrenalectomized bilaterally were compared with those of control animals at the electron microscopic level.

Materials and Methods

In the present study, ten adult male Sprague-Dawley rats, aged 8-10 weeks at the beginning of the experiment, were used. They were assigned to treatment or control groups on a random basis and were maintained under standardized conditions of light (12 hour on/12 hour off) and temperature (22±2°C). Food and water were freely available. Animals were anaesthetized with 25 mg/kg thiopental sodium. Bilateral adrenalectomy was performed by dorsal approach. Control animals (n=4) were sham operated with a skin and muscle incision and handling of the adrenal glands (10,11). To prevent contamination along the postoperative period, dissolved chloramphenicol sodium succinate flacon (Farmitalia Carlo Erba, Milan Italy) was sprayed onto the incision area. The skin incision was closed with 3-0 surgical sutures. Polyvinylpyrrolidone-iodine complex was used in the final cleaning of the skin. Adrenalectomized rats were given 0.9% saline to drink post-operatively (11). After 14 days, all animals were sacrificed by decapitation. Their pituitary glands were removed promptly. The tissue samples were fixed in 3% glutaraldehyde in 0.2 M phosphate buffer, postfixed in 2% phosphate-buffered osmium tetroxide, dehydrated in acetone, and embedded in Araldite CY 212. Semi-thin sections were cut with a Nova LKB Bromma ultratome, and stained with toluidine blue. Ultra-thin sections were stained with uranyl acetate and lead citrate, and examined in a Jeol 100 SX electron microscope (12,13).

Results

In the pars distalis of the hypophysis from control rats, corticotrophs were seen quite less in comparison with other endocrin cells. These corticotrophs were with pale cytoplasm and various shape; oval or angular in contour. Some of them had cytoplasmic processes. The granule content of corticotrophs could not be seen clearly under low magnification, whereas it was observed that the granules were often arranged in a single row along the cell membrane under higher magnification in the controls. Corticotroph granules were much smaller than those of mammotrophs, somatotrophs and gonadotrophs (Figure 1). Thyrotroph granules were also smaller than the granules of above-mentioned cells, but that they scattered within the cytoplasm differed thyrotrophs from corticotrophs. In the hypophysis of adrenalectomized rats, more corticotrophs were seen. These corticotrophs were more irregular in shape, and had more cytoplasmic processes than in controls. It was observed that these cytoplasmic processes extended between neighbor cells (Figure 2), and surrounded some of the other cells like octopus arms (Figure 3). The corticotrophs of controls were poor in granular endoplasmic reticulum (GER) and Golgi organelle. There were some round or cylindrical mitochondria. The secretory granules arranged in a single row under the plasmalemma were different in size and density from each other, but most of them were small and electron-lucent (Figure 4). It was determined that the secretory granules of corticotrophs in adrenalectomized rats were increased in number and size (Figure 5,6). They were arranged in 3-4 rows under the cell membrane in some spots, accumulated particularly in the cytoplasmic processes, and distributed throughout the cytoplasm (Figure 5,6). Variation in shape and electron-density of these secretory granules were obvious. There were numerous solid granules having various degrees of electron density, and many haloed granules which were vesicles containing a dark core of various sizes and densities (Figure 2,5,6). In addition, there were well-developed GER, numerous ribosomes,

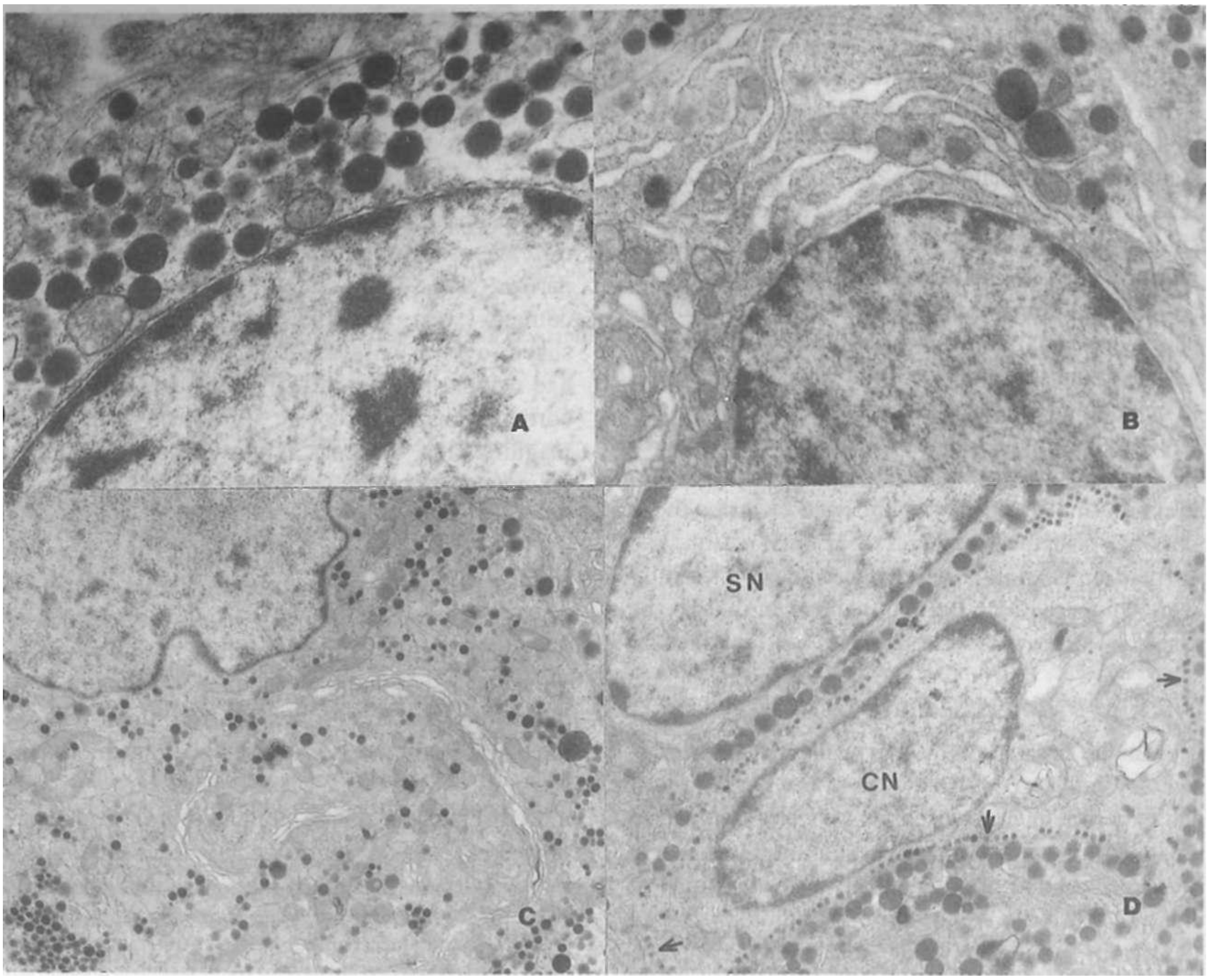

Figure 1. Electron micrographs from the hypophysis of a normal rat. A. A somatotroph, x 8300. B. A mammotroph (lactotroph), x 8300. C. A gonadotroph, x 6600. D. CN: the corticotroph nucleus; SN: the somatotroph nucleus; the arrows indicate the corticotroph's secretory granules arranged in a single row under the plasmalcmma, x 5000.

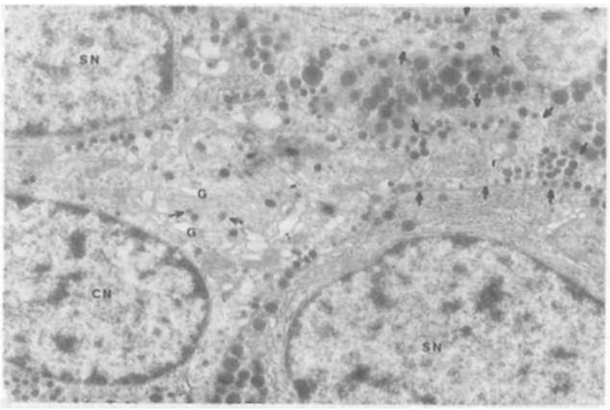

Figure 2. Electron micrograph from the hypophysis of an adrenalectomized rat. CN: the corticotroph nucleus; SN: the somatotroph nucleus; G: the Golgi organelle; r: ribosomes; single arrows indicate the cell processes extending between neighboring cells; double arrows indicate haloed granules, x 8300.

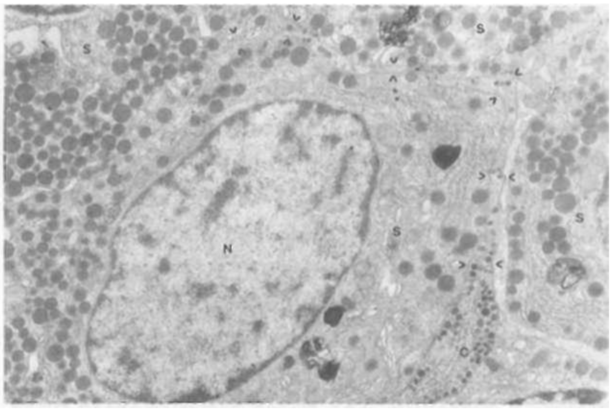

Figure 3. Electron micrograph from the hypophysis of an adrenalectomized rat. S: somatotrophs; N: the nucleus of a somatotroph; C: the corticotroph's cytoplasmic process surrounding other cells; arrowheads indicate the cytoplasmic process, x 6600.

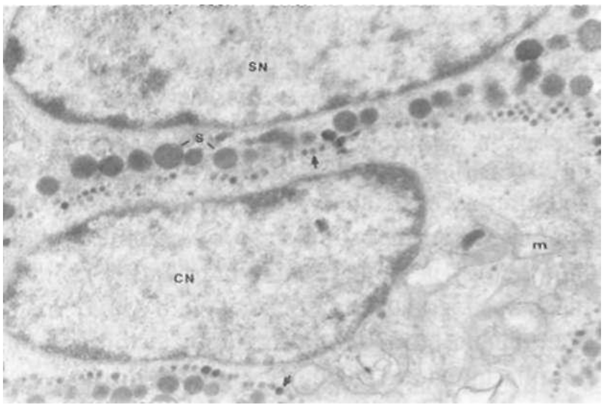

Figure 4. Electron micrograph from the hypophysis of a normal rat. CN: the corticotroph nucleus; SN: the somatotroph nucleus; the arrows indicate the corticotroph's secretory granules arranged in a single row under the plasmalemma; m: mitochondrion; S: somatotroph granules, x15000.

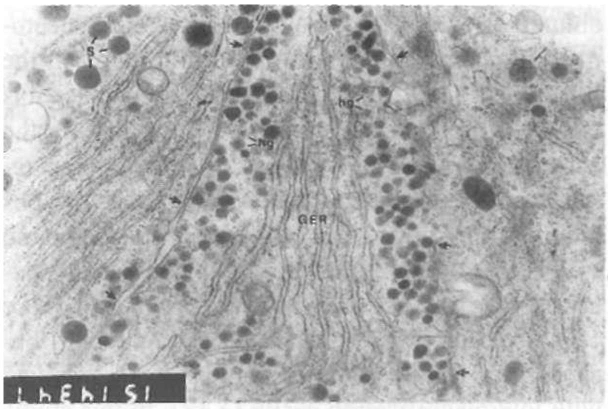

Figure 5. Electron micrograph from the hypophysis of an adrenalectomized rat. GER: granular endoplasmic reticulum; hg: haloed granules; S: somatotroph granules; the arrows indicate the cell membrane of the corticotroph. There are secretory granules arranged in 3-4 rows under the plasmalemma of corticotroph., x 15000.

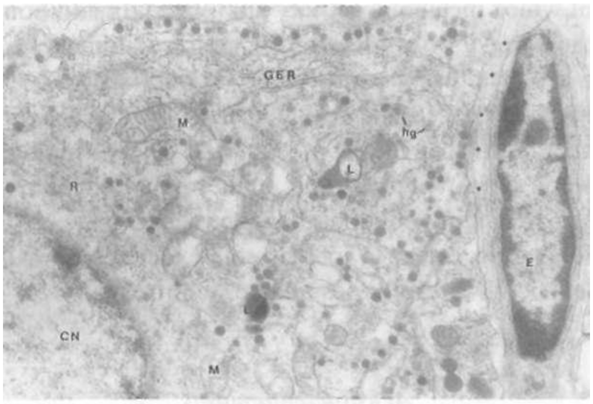

Figure 6. Electron micrograph from the hypophysis of an adrenalectomized rat. CN: the corticotroph nucleus; GER: granular endoplasmic reticulum; M: mitochondrion; R: ribosomes; L: lysosomes; hg: haloed granules; E: endothelial cell; asterisks indicate the contact surface between corticotroph and capillary, x 10000.

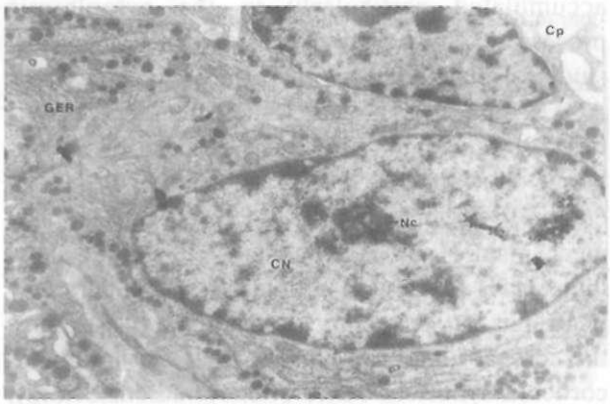

Figure 7. Electron micrograph from the hypophysis of adrenalectomized rat. CN: the corticotroph nucleus; Nc: nucleolus; GER: granular endoplasmic reticulum; Cp: capillary, x 8300.

many primary or secondary lysosomes and spherical or cylindrical mitochondria in the corticotrophs of adrenalectomized animals (Figure 6). GER was generally arranged in the form of parallel aggregations of cisternae (Figure 5,7). The Golgi organelle was also well-developed. It was determined that the Golgi organelle had multiple stacks of dilated cisternae, and there are many solid and haloed granules around the Golgi membranes (Figure 2). The corticotrophs of adrenalectomized rats had a round or oval nucleus containing a prominent nucleolus (Figure 7). Apart from these, it was also identified that the corticotrophs of adrenalectomized animals had a bigger area of contact with capillaries than those of controls (Figure 6,7).

Discussion

In this study, we observed few corticotrophs in the hypophysis of control animals, but far more in that of adrenalectomized ones. Erkocak have been reported that, the corticotrophs in normal hypophysis are less than the other endocrin cells (3). Some researchers showed that the corticotrophs are increased in number and size after bilateral adrenalectomy (1,14-18). Several scientists also reported that corticotrophin-releasing factor (CRF) secreted abundantly from hypothalamus after adrenalectomy stimulates the corticotroph-proliferation in the hypophysis (19,20). According to this evidence it is expected to observe more corticotrophs in the adrenalectomized rats than in controls. Some researchers were reported that normal corticotrophs are irregular in shape, with pale cytoplasm and small secretory granules tending to be located adjacent to the cell membrane (1,3,15,17), and these cells are often misidentified as chromophobes because their small granules could not be resolved with low magnification easily (1,17). In this study, even though the corticotrophs of controls were in known classical appearance, it was determined that the granules of corticotrophs in the adrenalectomized rats were increased in number and size, distributed throughout the cytoplasm, arranged in 3-4 rows along the cell membrane in some spots, accumulated particularly in the cytoplasmic processes. These data were supported by many studies declared before (14,15,17). It is known that ACTH induces the synthesis and secretion activities of the adrenal cortex. The glucocorticoids released from the adrenal cortex control ACTH secretion of hypophysis with the negative feed back mechanism (1-3). However, recent studies show that ACTH is secreted not only from pituitary corticotrophs but also from adrenal gland (4-9). Thus, the adrenal gland can control itself for the glucocorticoid synthesis. After bilateral adrenalectomy, it is expected that only corticotrophs take over the ACTH synthesis. Therefore, ACTH granules and the organelles required for the ACTH synthesis should be increased in these cells after adrenalectomy. Indeed in this study, quite well-developed GER and Golgi organelle, numerous ribosomes and a prominent nucleolus in addition to increased secretory granules were seen in the corticotrophs of adrenalectomized rats. As known, ACTH is a hormone, in the structure of polypeptide; GER and ribosomes are the organelles synthesizing proteins in the cells (1-3). Mostly proteins are synthesized as inactive proproteins, and then become mature proteins in the Golgi membranes or secretory granules (2). The prominent nucleolus suggests an increase in the metabolic activity of the cell (1-3). In control and adrenalectomized rats, the secretory granules of corticotrophs were different in size and density from each other. Particularly in the corticotrophs of adrenalectomized animals, many haloed granules and numerous solid granules having various degrees of electron density were observed. Kurosumi et al. reported that most of the secretory granules of normal corticotrophs are characteristically haloed, and after adrenalectomy, those cells are filled with a great abundance of secretory granules whose morphology is quite variable (21). In my opinion, the haloed granules associated with Golgi membranes in the corticotrophs of adrenalectomized rats may be immature secretory granules. In fact, in a cell whose synthesis- and secretion-activities increased, it is normal to find out immature secretory granules among the solid ones. Besides, in the corticotrophs of adrenalectomized animals, there were more lysosomes than in those of controls. The lysosomes increased in number could be used for the digestion of residual materials produced by the cells of which metabolic activity stimulated. In addition, the corticotrophs of adrenalectomized animals had more cytoplasmic processes extending between neighbor cells, and a bigger area of contact with capillaries than those of controls. These findings were supported by some studies reported before (16,18). In my opinion, the latter changes are helpful and necessary to increase the exchange of matters between capillaries and corticotrophs.

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