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Ultrastructural studies of adrenal adenoma causing primary aldosteronism

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Summary. Adrenal adenoma tissue was obtained from 7 patients with the diagnosis of primary aldosteronism and was studied electron microscopically. Spironolactone was administered in 6 of these patients, but not in the remaining patient. Most of the mitochondria of the tumour cells possessed tubular cristae, giving an appearance similar to the mitochondria in the cells of the zona glomerulosa. Spironolactone bodies were seen in the tumour cells of 6 patients who were given spironolactone preoperatively, but were not observed in these cells in the patient not given spironolactone. The literature on the developmental mechanism of this spironolactone body was reviewed.

Key words: Primary aldosteronism – Adrenal adenoma – Ultrastructure findings – Spironolactone bodies

Introduction

The intracellular ultrastructural characteristics of tumour cells from adrenal adenomas have been studied electron microscopically in patients with primary aldosteronism (Cervós-Navarro et al. 1965; Propst 1965; Beskid et al. 1978; Reidbord and Fisher 1969, Sommers and Terzakis 1970; Kano et al. 1979, Mazzocchi et al. 1982). No consensus has been reached on the developmental mechanism of the so-called spironolactone body. This inclusion body with myelin-like structure is seen in the tumour cells of patients treated with spironolactone. In this report, the ultrastructure of adrenal adenomas removed from 7 patients with primary aldosteronisms is presented, with descriptions of the spironolactone bodies.

Materials and methods

The specimens consisted of adrenal tumours removed from 7 patients with the clinical diagnosis of primary aldosteronism. Histological examination confirmed the diagnosis of adenoma. The age and sex of the patients as well as the size of the tumour are summarized in Table 1.

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Table 1. Data of seven patiens with adenoma

Case	Age	Sex	Size of tumour (mm)	Plasma K (mEq/l)	Plasma renin activity (ng/ml/hr)	Spironolactone therapy	
						Duration (days)	Total dosage (mg)
1	55	F	12×10	2.8	0.2	10	750
2	36	F	10×10	2.9	0.1	20	2,100
3	36	M	12×13	3.0	0.4	9	1,350
4	33	F	11× 7	2.5	0.36	8	675
5	33	M	15×10	2.8	0.6	8	600
6	40	M	13 ×7	2.3	0.2	7	525
7	37	F	12×11	3.5	0.3	0	0

Adrenals removed from 2 patients with renal carcinoma without clinical endocrine abnormalities served as controls.

The specimens were immediately subjected to prefixation in 3% glutaraldehyde solution buffered to a pH of 7.4 with 2% cacodylate at 4° C for 2 h, followed by postfixation in 1% cacodylate buffered osmic acid solution for 1 hour. After dehydration with ethanol, substitution with propylene oxide was carried out and the specimen was then embedded in epoxyresin. Ultrathin slices were prepared using the LKB II ultramicrotome. After double staining with uranyl acetate and lead hydroxide, the sections were examined under the electron microscope, type 100 CX produced by Nippon Denshi.

Results

I. Control group

Cells in the zona glomerulosa, zone fasciculata and zona reticularis generally showed similar fine structure, with some differences noted in the distribution of intracellular organelles.

The mitochondria of the cells in the zona glomerulosa appeared round or oval with tubular cristae (Fig. 1). The mitochondria of the cells in the zone fasciculata were frequently round, and larger in size than those in the zona glomerulosa.

Tubular and vesicular cristae or purely vesicular cristae were seen, with the latter more frequently observed (Fig. 2). Mitochondria of the zona reticularis were occasionally long and narrow but usually had the appearance of vesicular cristae. A marked morphological difference was noted between the mitochondria of the cells in the zona glomerulosa and of those in the zona fasciculata and zona reticularis, but no remarkable difference was noted between mitochondria of cells in the zona fasciculata and those in the zona reticularis.

II. Adenomata

Most of the nuclei of the adenoma cells appeared round, but nuclei with irregular shapes were occasionally seen. The presence of numerous vacuoles

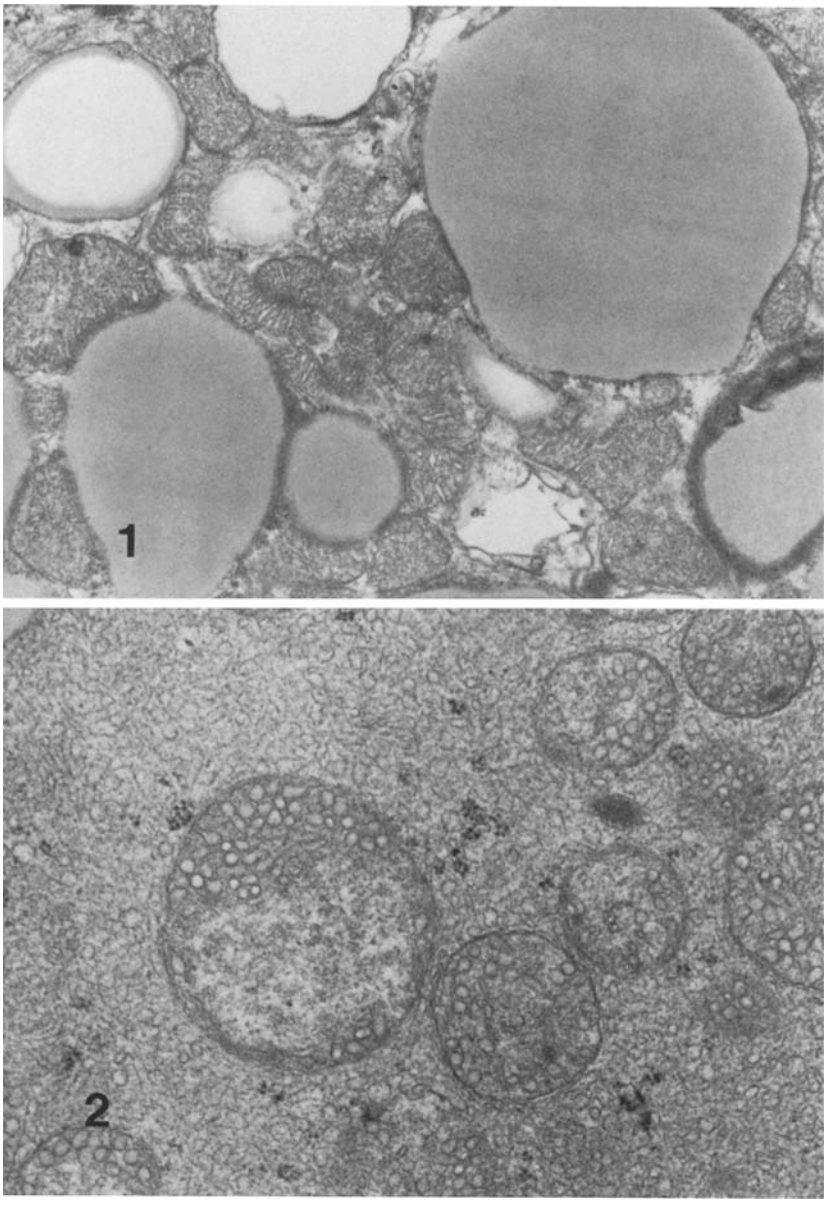

Fig. 1. A cell in the zona glomerulosa. Mitochondria are round or oval, with tubular cristae. × 20,800

Fig. 2. A cell in the zona fasciculata. Mitochondria are round or oval, mainly containing vesicular, and occasionally tubular, cristae. × 33,300

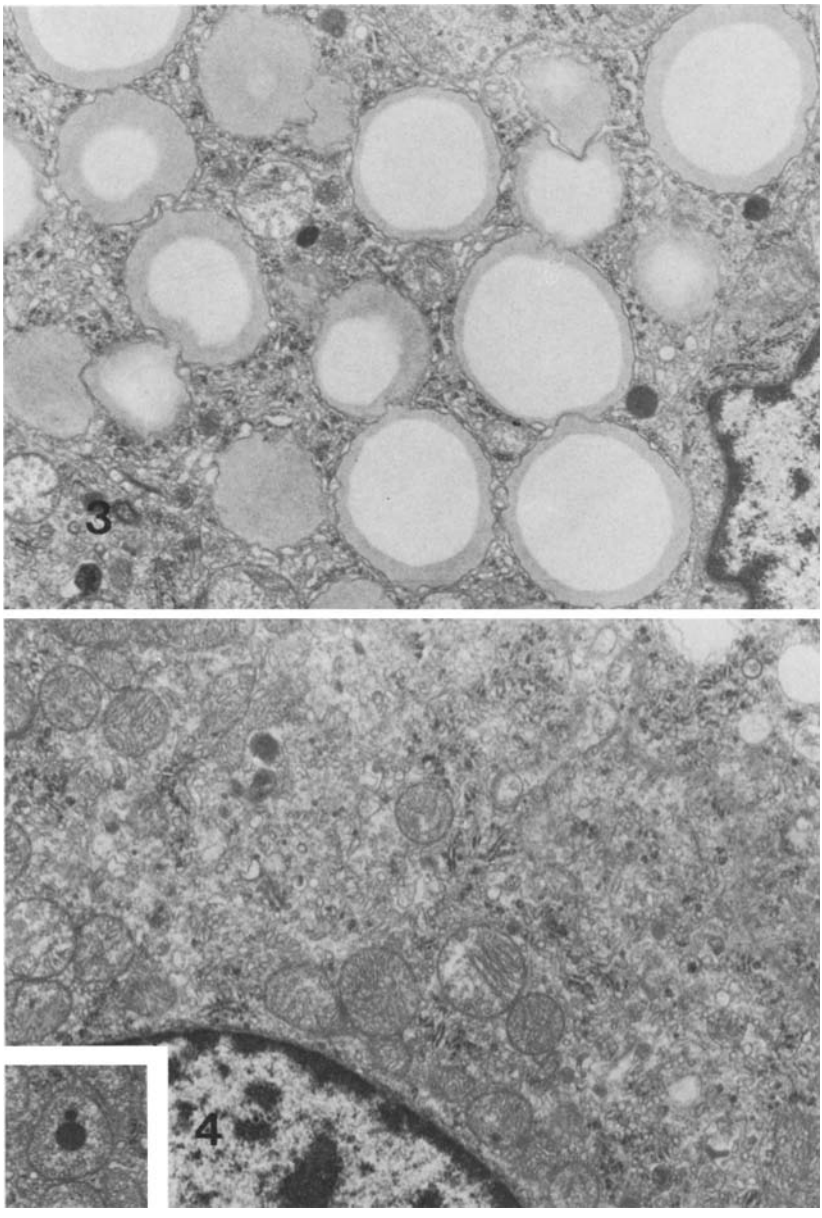

Fig. 3. An adenoma cell with numerous vacuoles, surrounded by smooth surfaced endoplasmic reticulum with vesicular appearance. Free ribosomes were also noted. ×16,800

Fig. 4. An adenoma cell. Mitochondria contained tubular cristae. (*Insert*) Materials with high electron density were occasionally noted within the mitochondrial matrix. ×15,600

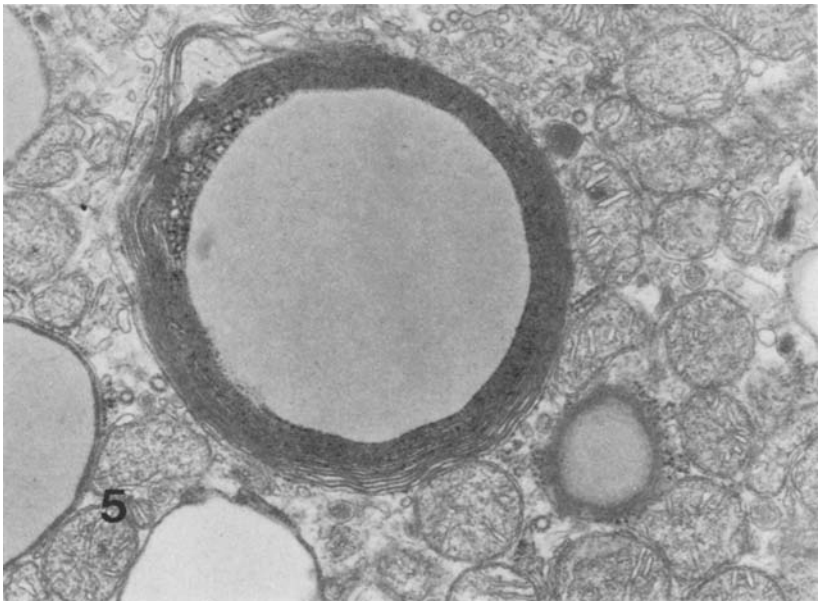

Fig. 5. An adenoma cell from a patient given spironolactone preoperatively. An inclusion body consisting of concentric and whirl-like smooth membranes, the so-called spironolactone body, is noted. × 26,000

occupying a large part of the cytoplasm characterized the tumour cells. The size of these vesicles varied greatly. In some portions, a tight mutual contact between the vacuoles was noted, but no fusion was found (Fig. 3).

Smooth surfaced endoplasmic reticulum was extremely well developed, with vesicular or tubular structure. The rough surfaced endoplasmic reticulum was poorly developed except in a few cases. In cases in which it was well developed, several parallel layers were formed.

Numerous mitochondria were noted. Their cristae were mostly tubular in shape in a lamellar arrangement (Fig. 4). Some of these were vacuolar or mixed tubular-vacuolar in appearance, while many others were round or oval in shape, and some long and narrow. Within the mitochondrial matrix, a teardrop-like material with high electron density was occasionally noted.

Inclusion bodies surrounded by smooth membranes of concentric, whirl-like structure were noted, and a lipid droplet was seen at the center (Fig. 5). Occasionally, fingerprint-like structures were noted. Such inclusion bodies were only seen in cases in which spironolactone was administered preoperatively.

Smooth surfaced endoplasmic reticulum with a vesicular appearance was developed around the vacuole. The degree of development was not dependent upon the administration of spironolactone.

Discussion

Aldosteronomas have been classified into three groups, the zona glomerulosa type (Cervós-Navarro et al. 1965; Kano et al. 1979), the zona fasciculata type (Propst 1965; Sommers and Terzakis 1970) and the hybrid type (Reidbord and Fisher 1969, Beskid et al. 1978; Mazzocchi et al. 1982). One similarity between the tumour cells and the adrenocortical cells consists in the shape, size and inner structure of mitochondria.

The tumour cells contained a few different types of mitochondria dependent upon the site of origin. Most of these mitochondria were round with tubular cristae, resembling those of the zona glomerulosa. Based on these findings, adenoma cells are thought to be derived from the cells of the zona glomerulosa. Similar findings were obtained in the central and peripheral portions of the adenoma. Fisher and Horvat (1971), however, reported in their experiments with rats that the morphology of the mitochondria changed with the stimulation or inhibition of aldosterone production. This may represent one of the reasons for the variable reports by different investigators on the origin of the tumour cells.

In cases in which spironolactone had been administered preoperatively, structures consisting of concentric, whirl-like structures with a smooth double membrane containing a central lipid droplet at the core were found in many adenoma cells. These are the spironolactone bodies reported by Janigan (1963) and many investigators. Several investigators have reported the appearance of these bodies in the cells obtained by autopsy in the zona glomerulosa from patients administered spironolactone during their lifetime and also in patients with aldosterone producing tumours (Jenis and Hertzog 1969; Davids and Medline 1970, Fisher and Harvat 1971; Okano et al. 1972; Kovacs et al. 1973). According to others, these structures appeared only in the adenoma cells of aldosterone-producing tumours (Cain et al. 1974; Shrago et al. 1975; Conn and Hinerman 1977; Kano et al. 1979).

It is well known that aldosterone is synthesized in the zona glomerulosa of the adrenal cortex and that spironolactone, an aldosterone antagonist, antagonizes aldosterone and inhibits its action in the target organs. Spironolactone has also been reported to act directly on the adrenal to inhibit aldosterone synthesis (Erbler 1972; Erbler 1973; Sundsfjord et al. 1974).

In the present study, those spironolactone bodies were found in most of the adenoma cells from patients who had been treated with spironolactone, but none were seen in the adenoma cells from patients who were not treated with the drug, thus the inclusion body is related to spironolactone administration, and is not a characteristic of the adenoma cells.

According to Conn and Hinerman (1977), spironolactone bodies are a morphological expression of an inhibitory action on aldosterone biosynthesis by the drug. The bodies consist of smooth membranes giving a whirl-like structure. Some steroid-secreting cells are known to have smooth surfaced endoplasmic reticulum with whirl-like structure (Christensen 1965; Christensen and Fawcett 1966; Blanchett 1966; Enders and Lyons 1964; Giacomellie et al. 1965) so that these findings are probably due to the thick-

ening and proliferation of such smooth surfaced endoplasmic reticulum (SSEM). According to Jenis and Hertzog (1969) and Davis and Medline (1972), peripheral antagonism to aldosterone by spironolactone starts a negative feedback mechanism. Production of aldosterone and cholesterol increases via the renin-angiotensin system. As a result, the SSEM, the site of biosynthesis of these substances proliferates and forms spironolactone bodies. Jenis and Hertzog (1969), moreover, demonstrated a continuity between these bodies and the SSEM. In our studies, no continuation was found between spironolactone bodies and the SSEM and its extent was similar whether or not there was preoperative administration of spironolactone. From these findings, it is unlikely that the bodies were formed due to an increase in SSEM. It is, however, impossible to rule out the possibility that the spironolactone bodies developed *from* SSEM.

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