Histol Histopathol (2002) 17: 185-190

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Chronic hypoxia induced ultrastructural changes in the rat adrenal zona glomerulosa

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Summary. The adrenal cortex plays an important role in adaptation to various forms of stress, including hypoxia. While physiological changes in the aldosterone metabolism during hypoxia have been extensively described, few studies have focused on the morphological changes in the adrenal glands under chronic hypoxia.

We studied the ultrastructure of the *zona glomerulosa* of 6-month-old Wistar rats exposed to chronic normobaric hypoxia. Animals were divided into two groups: control (n=12) and hypoxic (n=12). In this latter group, the animals were kept at 7% O_2 concentration after a gradual adaptation (21, 15, 12, 10, 8, 7 vol% O_2). The duration of the study was 112 days.

In comparison with normoxic rats, body weight and adrenal gland weight of hypoxic animals was significantly reduced by 18.5% (p=0.006) and 14.7% (p=0.001) respectively. The thickness of the *zona glomerulosa* decreased due to atrophy of cells. The main ultrastructural changes observed were: 1) a decrease in, or complete elimination of, lipid droplet content; 2) a marked increase in lysosome number; and 3) the presence of giant mitochondria.

Our findings show that rats fail to adapt to severe chronic hypoxia. The ultrastructural changes in the *zona glomerulosa* found in the present study could reflect changes in the aldosterone pathway.

Key words: Chronic hypoxia, Zona glomerulosa, Ultrastructure, Rat, Mitochondria

Introduction

Chronic hypoxia occurs in humans and animals either under normal atmospheric pressure (normobaric conditions) or under hypobaric conditions (e.g. during climbs to high altitudes and, occasionally, high-altitude flights). An advantage of normobaric hypoxia studies is that secondary effects of low atmospheric pressure do not confuse the effects of hypoxia. Previously, we developed a chronic normobaric hypoxia model in the conscious rat (Hamdorf and Cervós-Navarro, 1990) which presents similarities to cardiopulmonary and endocrine adaptations to normobaric hypoxia in humans.

The adrenal cortex plays an important role in adaptation to various forms of stress, including hypoxia. The physiological changes of aldosterone metabolism in hypoxia have been extensively studied (Lawrence et al., 1990; Raff and Jankowski, 1993, 1994; Hillier et al., 1997). However, relatively few studies have assessed morphological changes of the adrenal glands during chronic hypoxia. This lack of information constitutes an important gap in the body of knowledge concerning acclimatisation to high altitude. Wolman et al. (1993) studied the adrenal glands of rats exposed to chronic normobaric hypoxia using light microscopy and found pathological changes in the *zona glomerulosa*. The purpose of the present study was to examine the ultrastructure of the *zona glomerulosa* of the adrenal cortex in a model of chronic normobaric hypoxia (Hamdorf and Cervós-Navarro, 1990).

Materials and methods

Animals and experimental conditions: hypoxic model

Twenty-four male Wistar rats, 6±0.5 month were used. Animals were housed in Nalgene cages (2 per cage) with a batch of six cages placed in Plexiglas chambers (0.36 m^3 in volume). A total of two Plexiglas chambers were used.

The animals were divided into two groups: hypoxic and normoxic. In the chamber housing the hypoxic group, the O_2 concentration was gradually decreased to 7% (vol) by increasing the N_2 flux to the chamber to 19.5 l/min during the experiment (Table 1). The duration of each step and the constitution of the gas mixture were

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chosen on the basis of earlier experiments (Hamdorf and Cervós-Navarro, 1990). Gas mixtures containing different concentrations of O_2 and N_2 were pumped into the chambers at a rate of 1800 l/hour. Flowmeters regulated the flow of N_2 and air in the mixture. The gas concentrations (including CO_2) were monitored and kept constant with the aid of an "Oxytest" oxymeter and a CO_2-meter "Uras M" (Hartmann and Braun AG, Frankfurt). Throughout the experiment, the control group was maintained under the same conditions as above, but for the presence of normoxia in the chamber. The whole gas volume in the chambers was replaced 5 times per hour. The animals were on a 12-hour light (6 am to 6 pm) to dark cycle. For cleaning and feeding, the chambers were opened every 3 days for a few minutes. The duration of the study was 112 days.

All procedures complied with the German laws for protection of animals, and were approved and registered.

Light and electron microscopy

At the end of the experimental period, animals were anaesthetised with an intraperitoneal injection of ketamine (100 mg/Kg) and 1 ml/Kg xylazine subcutaneously, and then perfused with a glutaraldehyde-based fixative (2% glutaraldehyde, 2% paraformaldehyde in 0.2 mol/L sodium cacodylate buffer, pH 7.4). The adrenal glands were immediately removed and weighed. Several blocks of 1 to 2 mm^3 per selected gland area were prepared and kept in the same fixative for 12 hours. Tissue blocks were rinsed and post-fixed in a cacodylate-buffered solution of 1% OsO_4 for 120 minutes, followed by dehydration in a graded series of acetone solutions and embedding in Araldite.

Semithin sections of 1 µm were stained with Toluidine blue. Ultrathin sections were examined in a ZEISS EM-10 transmission electron microscope at 80Kv.

Statistical analysis

Data are expressed as mean ± standard deviation. The statistical comparison of the data was done by Student's t-test. $P<0.05$ was considered significant.

Table 1. Experimental conditions of the animals in the hypoxic and control groups.

GROUP	$[O_2]$ (vol%)	AIR SUPPLY (l/min)	$[N_2]$ (l/min)	DAYS	ALTITUDE (m)
Control group	21	30	0	112	
Hypoxic group	21	30	0	7	
	15	22.5	7.5	7	2,600
	12	18	12	21	4,450
	10	15	15	21	5,800
	8	12	18	28	7,400
	7	10.5	19.5	28	8,350

Atmospheric pressure: 759±4 mmHg; Temperature: 22± 2 °C; Humidity: 60%.

Results

Body weights and adrenal gland weights

At the end of the experiment, hypoxic animals showed a significantly lower body weight (18.5%; p=0.006) and lower adrenal gland weight (14.7% ; p=0.001) in comparison with controls (Table 2).

Light microscopy

Control group

The *Zona glomerulosa*, which is immediately beneath the capsule, was composed of 8-10 layers of cells. The glomerulosa cells were well outlined. The nuclei were round or oval, and the nucleo-cytoplasmic ratio was high. The cytoplasm was usually finely vacuolated due to the high lipid droplet content. Sinusoids were observed between the cells.

Hypoxic group

In all animals, the *Zona glomerulosa* was composed of 6 layers of cells. The nucleo-cytoplasm ratio of the glomerulosa cells was higher in comparison to normoxic rats. The cytoplasm was more intensely stained, and relatively few lipid vacuoles were detectable (Fig. 1). Lipofuscin granules were sometimes found in the cytoplasm, particularly in cells situated at the border with the *zona fasciculata*. The profile of nuclei was generally oval and they were smaller and more intensely stained than in the control group.

Electron-microscopic findings

Control group

The major diameter of the glomerulosa cells was 14±2 µm. The cytoplasm contained a large number of round lipid droplets (Fig. 2) with a matrix of almost complete dark density. Sizes of lipid droplets ranged from 0.5 to 2 µm. Some scattered lysosomes were observed. Mitochondria were mostly round or oval in section and their wideness measured 0.66 µm (SD±0.05). Mitochondrial matrix was relatively electron-dense and contained tubular cristae. The

Table 2. Number and weights in control and hypoxic rats.

GROUP	BODY WEIGHT (g)	ADRENAL WEIGHT (g)
Control (n=12)	480.5±4.5	0.41±0.01
Hypoxic (n=12)	390±27 *	0.35±0.02**

Values are expressed as means±SD. Statistical significance $p< 0.05$. *: p=0.006; **: p=0.001

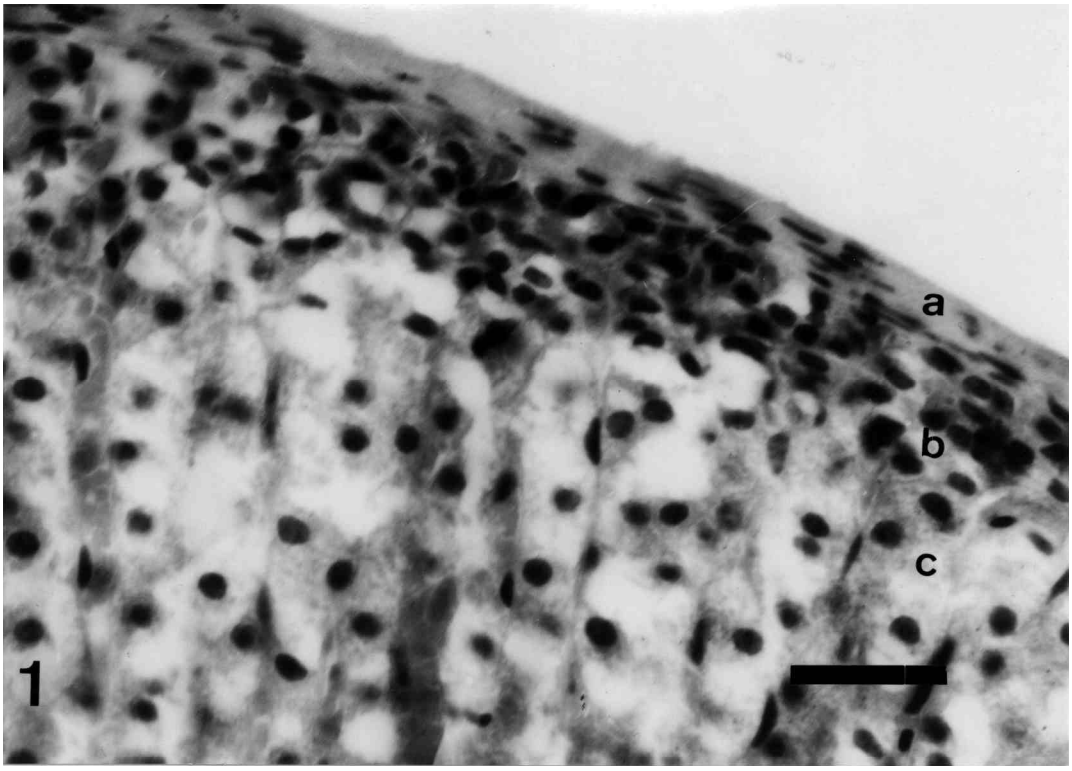

Fig. 1. Hypoxic group. Atrophy of the zona glomerulosa with reduction of cell cytoplasm. a: capsule, b: zona glomerulosa, c: zona fasciculata. H&E. x 500. Bar: 40 µm.

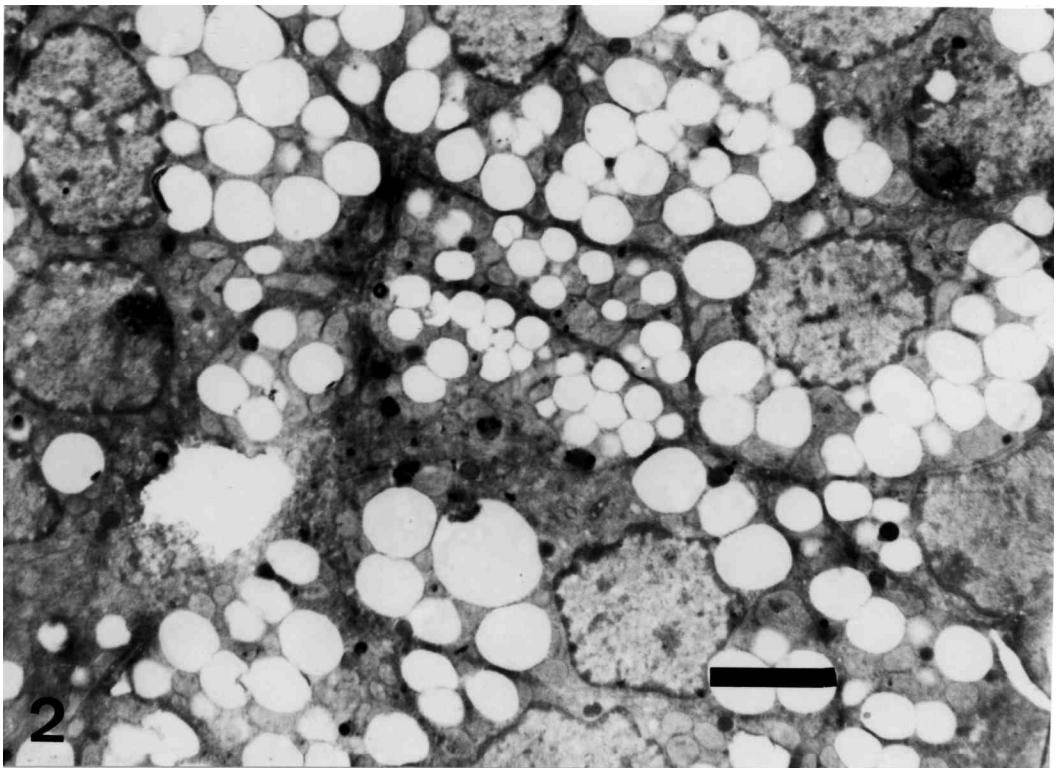

Fig. 2. Control group. Abundant lipid droplets distributed in all cells. x 4,200. Bar: 4 µm.

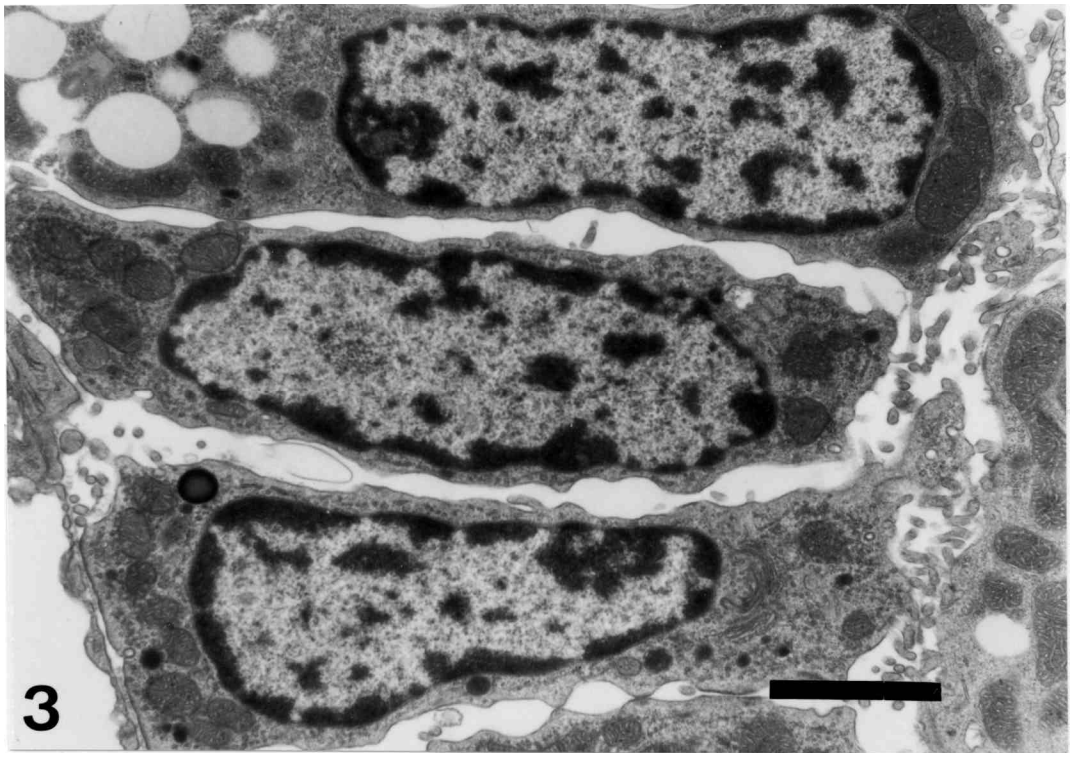

Fig. 3. Hypoxic group. Decrease (or complete disappearance) of lipid droplets. x 11,000. Bar: 2 µm.

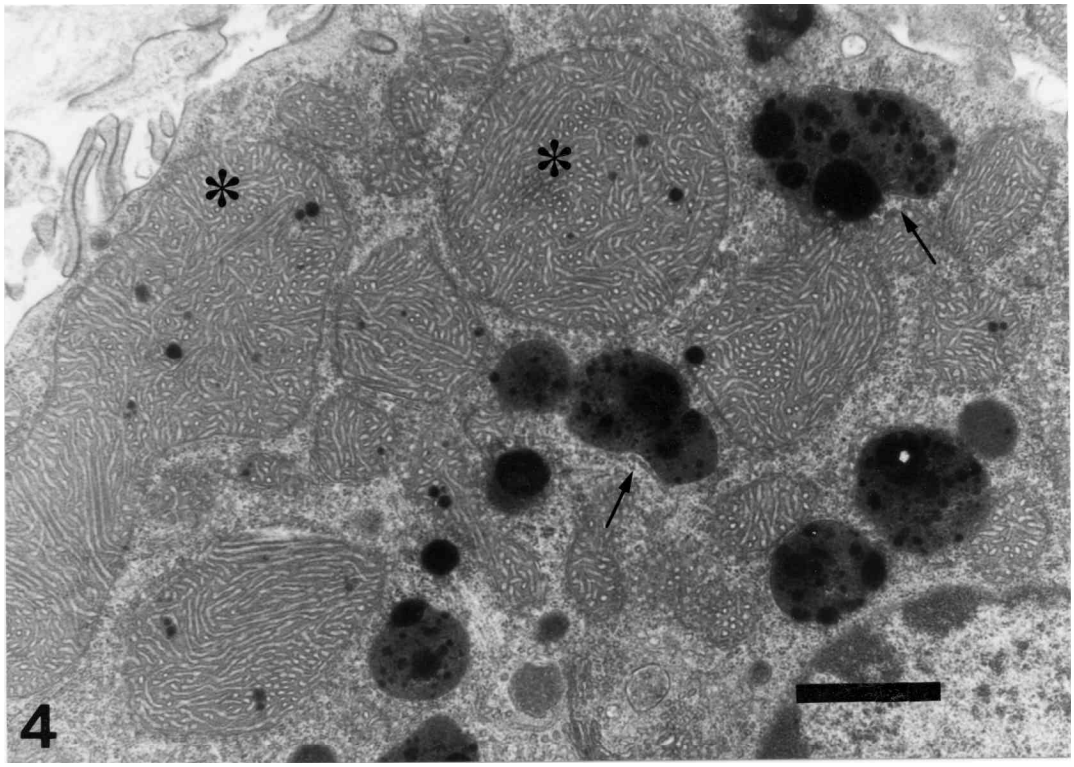

Fig. 4. Hypoxic group. The number of lysosomes (arrows) is markedly increased. These are lipofuscin bodies. Giant mitochondria (asterisk) can be seen and heterochromatin is increased in nuclei. x 17600. Bar: 1 µm.

nucleus showed an irregular round shape uniformly filled with chromatin and a nucleolus (Fig. 2). The diameter of the nuclei ranged from 5 to 7 mm.

Hypoxic group

Cells of the zona glomerulosa showed a significant reduction in size in comparision with controls ($p<0,05$). Their mean diameters were 10±2 µm. They were mostly oval and frequently had irregular processes. Their cytoplasm was reduced in size. The number of lipid droplets was scanty or they were absent (Fig. 3). There was a marked increase in the number of lysosomes. The mitochondrial wideness was significantly larger (0.76±0.08 µm) in comparision with controls ($p<0,05$). Occasional giant mitochondria with diameters ≥ 3,5 µm were observed (Fig. 4). Some had a normal structure, while others showed clusters of cristae arranged peripherically. Nuclei were predominantly oval, and the major diameter ranged from 4 to 6 µm. In some cells heterochromatin was increased.

Discussion

The morphological effects of experimental hypoxia vary depending on duration: acute (minutes), subacute (hours) and chronic (days), and the degree of hypoxia: mild (O_2 -14.5%), moderate (O_2 -10%), and severe (O_2 <10%). Such studies have been carried out in several animal models: dogs (Raff et al., 1983), rats (Raff and Chadwick, 1986), cows (Raff and Jankowski, 1993), sheep (Curran-Everett et al., 1988) as well as in humans (Heyes et al., 1982; Milledge and Catley, 1982; Raff et al., 1983). However, to our knowledge, none of these studies included an experimental model of severe chronic normobaric hypoxia. Therefore, our results are not easy to compare with those of other reports.

In hypoxic animals, body weight was significantly reduced (18.5% - $p=0.006$) in comparison to normoxic controls, a figure similar to that reported by other authors (Hamdorf and Cervós-Navarro, 1991; Wolman et al., 1993; Cervós-Navarro et al., 1999). This difference may be due to atrophy of muscles and reduction of body fat. The atrophy of muscles is secondary to reduced locomotor activity, which is a sensitive parameter in acute and chronic hypoxia (Hamdorf and Cervós-Navarro, 1991) and may be due to decreased activity. The marked reduction of body fat may result from reduced food intake, attributed to a reduction in neurotransmitter synthesis in the corpus striatum and substantia nigra; neurotransmitters play an important role in regulating eating and drinking behaviour (Hamdorf and Cervós-Navarro, 1991).

Our results show a smaller difference in adrenal gland weight (14.75% less) than in overall body weight (18% less). Some authors such as Mohri et al. (1983), who studied rats exposed to 12.5% O_2 for a period of 30 min-14 days, and Gosney (1985), who studied rats exposed to a barometric pressure of 380 mm Hg (equivalent to an altitude of 5500 m) for 28 days, described a marked increase in the adrenal gland weight. They attributed this increment to a hyperplasia of the cortex and medulla. In our conditions, the loss of adrenal weight appears to be due to a decreased width of the *zona glomerulosa*, as the cortex occupies approximately 85% of the overall volume of the gland. In our report, we provide ultrastructural confirmation of light microscopic studies that have shown a reduction of the outer zone of the adrenal cortex in response to chronic hypoxia, leading to qualitative and quantitative decrease of cells, and finally atrophy (Wolman et al., 1993). The latter authors also described a hyperplasia of the adrenal medulla in rats exposed to 7% O_2, which they suggest is the cause of the smaller weight loss in the adrenal gland than in the body as a whole.

The *zona glomerulosa* seems to possess two features that render it particularly sensitive to oxygen tension: 1) the presence of 18 hydroxylase enzyme (mitochondrial enzyme) (Ray, 1980) or the ionic and/or osmotic sensitivity which are inherent to the zona glomerulosa but not fasciculata cells (Raff et al., 1990); and 2) the higher levels of PO_2 in the zona glomerulosa than in the deeper zones of the adrenal cortex, which means that small changes in oxygen delivery in vivo may have a profound effect on this zone (Colice and Ramirez, 1986; Raff et al., 1986).

Our most striking finding was the reduction in size and number of lipid droplets in the cells of the zona glomerulosa. This may be related to the overall reduction in lipid throughout the body. However, as observed in cases of acute (Andreis et al., 1990; Domenici-Lombardo and Cortesini, 1990; Coleman et al., 1995) or chronic stress (Ray, 1980), it may be due to the utilisation of the cholesterol stored in the lipid droplets in steroid synthesis. Since cholesterol is the main precursor of steroid hormone biosynthesis, and since 75-80% of cholesterol in the rat adrenocortical cell is contained in the lipid droplets (Ray, 1980), a decrease in the number of the latter would indicate a rapid conversion of cholesterol into pregnenolone.

In certain physiological experimental and pathological circumstances, lysosomes accumulate unesterified and/or esterified cholesterol, and so lipid-lysosome complexes appear to be involved in cholesterol homeostasis, by altering lipid compartmentalisation (Tóth et al., 1997). We observed an increase in lysosome number, similar to the findings of others in the sympathetic ganglia of rats exposed to chronic hypoxia (Chairkin et al., 1997) and the lung and liver of rats during induced acute circulatory hypoxia (Seredenko et al., 1992). Physiological studies have demonstrated that in stress cases, there are changes in acid phosphatase activity, an enzyme marker of lysosomes (Seredenko et al., 1992).

We occasionally observed giant mitochondria, with diameters up to 3 µm. To our knowledge, no megamitochondria have been reported before in the zona glomerulosa of rats exposed to chronic hypoxia. They

have been described in the hearts of rats exposed to 7% O_2, but not in those exposed to 8% O_2 (Cervós-Navarro et al., 1999). This variability in size was also observed in other zones of the cortex (reticularis and fasciculata) after a unilateral adrenalectomy (Ray, 1980) in aged rats (Murakoshi et al., 1985) and iron-deficient rats (Coleman et al., 1995).

The giant mitochondria showed a few peripheral cristae, as observed in pancreatic exocrine cells of rats after ethanol administration (Tandler et al., 1996). They may reflect a failure to adapt to chronic severe hypoxia. In conclusion, the ultrastructural changes found in the zona glomerulosa in the present study may reflect changes in the aldosterone pathway.

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Accepted October 22, 2001