*J. Anat.* (1981), **132**, 2, pp. 235–242
*With 4 figures*
*Printed in Great Britain*



# Effects of angiotensin II on the zona fasciculata of the rat adrenal cortex: an ultrastructural stereologic study

GASTONE G. NUSSDORFER, CLAUDIA ROBBA, GIUSEPPINA MAZZOCHI AND PIERA REBUFFAT

*Department of Anatomy, University of Padua, Via Gabelli* 65, 35100 *Padua, Italy*

(*Accepted* 8 *July* 1980)

## INTRODUCTION

It is now well known that the renin-angiotensin system stimulates the growth and the steroidogenic capacity of the adrenal zona glomerulosa (Fisher & Horvat, 1971; Hashida & Yunis, 1972; Kasemsri & Nickerson, 1976; Rebuffat *et al.* 1979; Mazzocchi *et al.* 1981). However, little attention has been paid to the effects of angiotensin on zona fasciculata cells (Nussdorfer, 1980). Fisher & Horvat (1971) did not find evident changes, whereas Maruyama (1972 and Tsuchiyama, Sugihara & Kawai (1972) described, in zona fasciculata cells of chronic hypertensive rats, increase in the smooth endoplasmic reticulum (SER) and hypertrophy of the Golgi apparatus. More recently Kasemsri & Nickerson (1976) demonstrated that chronic renal encapsulation-induced hypertension provokes in the rat zona fasciculata a significant increase in the volume of cells, nuclei, SER and lipid droplets.

It, therefore, seemed worth while investigating by stereological methods the effects of a chronic treatment with angiotensin II on the zona fasciculata of the rat adrenal cortex.

## MATERIALS AND METHODS

Thirty six young male albino rats (Wistar-derived) of about 200 g were divided into six equal groups, five of which received at 10.00 am daily ip injections of 1 mg–kg of angiotensin II (Sigma Chemical Company, St Louis, U.S.A.) in 0·5 ml of 0·85 % saline for 3, 6, 9, 12 or 15 consecutive days, respectively. The other group served as a control and received daily ip injections of 0·5 ml of 0·85 % saline for 15 consecutive days. To prevent the possible interference of the angiotensin II and/or the chronic stress, related to the repeated ip injections, with the hypothalamo-hypophyseal-adrenal axis, during the entire experimental period, all the rats were given daily ip injections of 2 mg/kg of dexamethasone (Decadron, Merck, Milan, Italy) and daily sc injections of 2 IU/kg of ACTH (Acthar gel, Armour-Erba, Milan, Italy). In fact, dexamethasone treatment conceivably suppresses ACTH release by the pituitary gland, whilst ACTH administration ensures the normal maintenance of adrenal growth. The animals were maintained on Purina rat-mouse chow and tap water *ad libitum* and were killed by cervical dislocation at 11.00 a.m.

Adrenal fragments of each rat were fixed in 3 % glutaraldehyde in 0·1 M-cacodylate buffer (pH 7·2), post-fixed in 1 % $OsO_4$ in 0·1 M phosphate buffer (pH 7·1) and embedded in an epoxy resin. Some glutaraldehyde-fixed fragments were sectioned at 50 $\mu$m with a TC2 Sorvall and the slices were incubated in Gomori's medium to demonstrate acid phosphatase activity (Miller & Palade, 1964). Thick sections were

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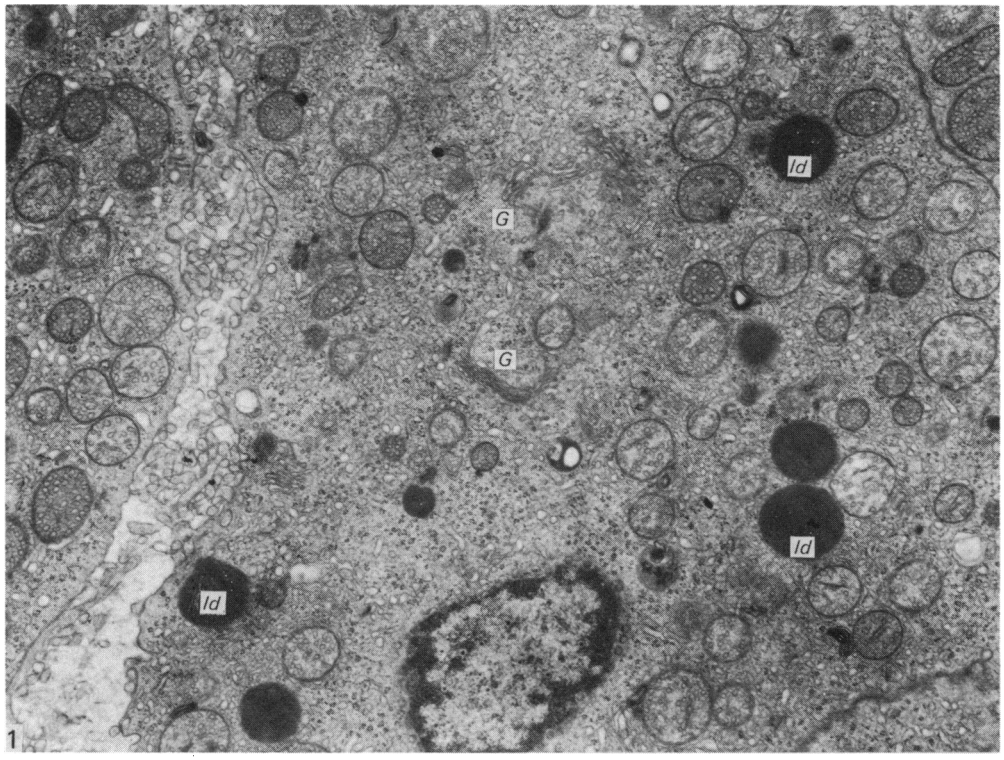


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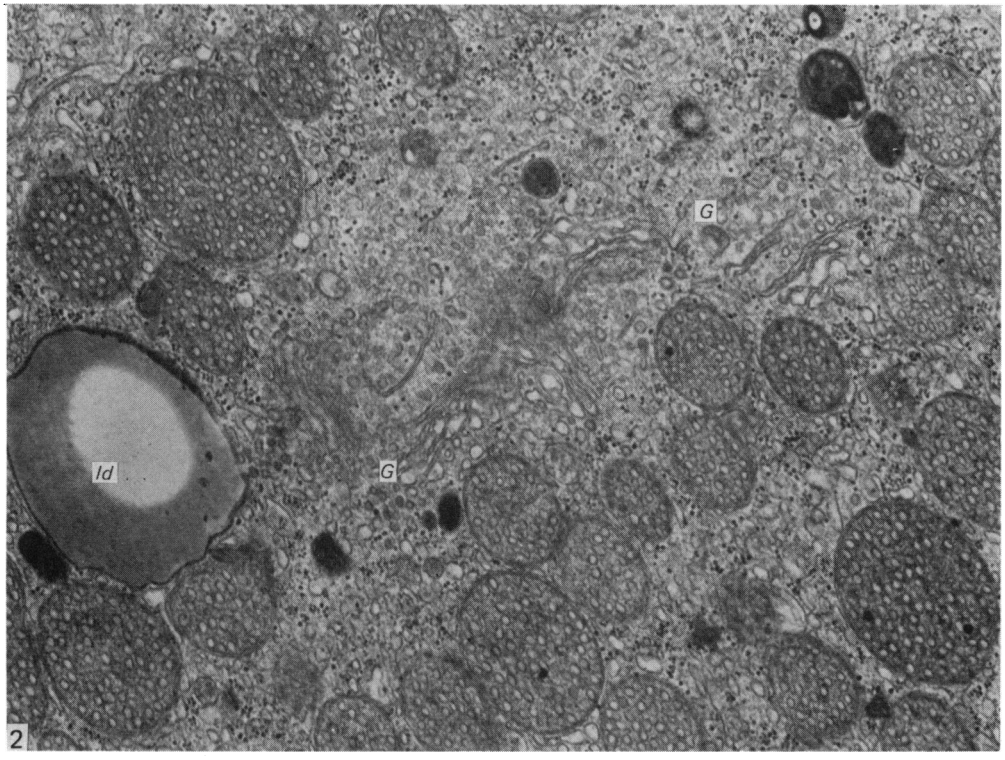


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made with LKB III ultramicrotomes and examined by light microscopy to select the middle portion of the zona fasciculata. Thin sections were counterstained with lead-hydroxide (Karnovsky, 1961) and examined in a Hitachi HU-12 electron microscope.

For the morphometric assessments the sampling procedure used was that described elsewhere (Nussdorfer, Mazzocchi & Rebuffat, 1973). The average cell volume and the absolute amount of the various organelles per cell were calculated according to Nussdorfer (1970), employing conventional stereological procedures (Weibel, 1969). The data obtained from each rat were averaged per experimental group and the standard error of the mean was calculated. Student's *t*-test was used for the statistical comparison of the data.

## RESULTS

The ultrastructure of the rat zona fasciculata has been described in several previous papers (eg. Idelman, 1978; Nussdorfer, Mazzocchi & Meneghelli, 1978), and therefore it will be only summarized here. Zona fasciculata cells (Fig. 1) contained round or ovoid mitochondria with vesicular cristae, a well developed smooth endoplasmic reticulum, some lipid droplets and a moderately large Golgi apparatus; a few acid phosphatase-positive dense bodies of various size were also observed.

In rats chronically administered with angiotensin the volume of zona fasciculata cells and nuclei was found to increase significantly in relation to the duration of treatment (Table 1). Very slight ultrastructural qualitative changes were noted: the juxtanuclear Golgi apparatus appeared hypertrophic and contained abundant vesicles (Fig. 2), and dense bodies were noticeably increased in number (Fig. 3). Dense bodies invariably displayed cytochemically demonstrable acid phosphatase activity (Fig. 4).

From a stereologic point of view (Table 1), it was found that the volume of the mitochondrial and lipid compartments as well as the surface area of mitochondrial cristae and smooth endoplasmic reticulum tubules were significantly increased with the number of days of angiotensin treatment. With the exception of the volume of the lipid compartment, the increases of the various morphometric parameters became significant only after 6 days of continuous treatment (Table 1). The volumes of the Golgi apparatus and the acid phosphatase-positive dense bodies were not stereologically evaluated.

## DISCUSSION

The present findings indicate that chronic angiotensin treatment induces increase in the volume of the rat zona fasciculata cells, which is almost exclusively due to the hypertrophy of the smooth endoplasmic reticulum and mitochondrial compartments. This datum fits well with the abundant biochemical evidence that the enzymes involved in the synthesis of corticosterone, the main steroid hormone secreted by rat adrenal zona fasciculata (Sandor, Fazekas & Robinson, 1976), are located in both these organelles (Tamaoki, 1973). In earlier works (Nussdorfer *et al.* 1978) it was

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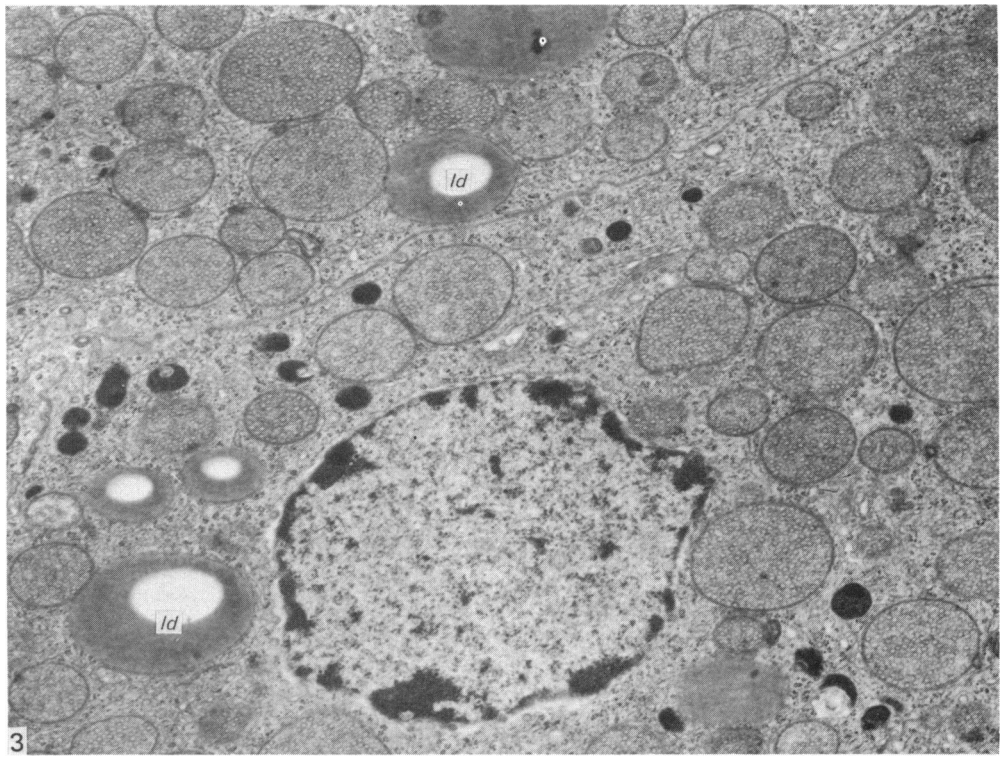
Fig. 1. Low power electron micrograph of a zona fasciculata cell of a control rat. Round mitochondria contain vesicular cristae, smooth endoplasmic reticulum tubules and free ribosomes are abundant, and few lipid droplets (*ld*) can be observed. *G*, Golgi apparatus. × 6000.

Fig. 2. Zona fasciculata cells of a rat after 6 days of continuous angiotensin treatment. The Golgi apparatus (*G*) is enlarged. *ld*, lipid droplet. × 21 000.

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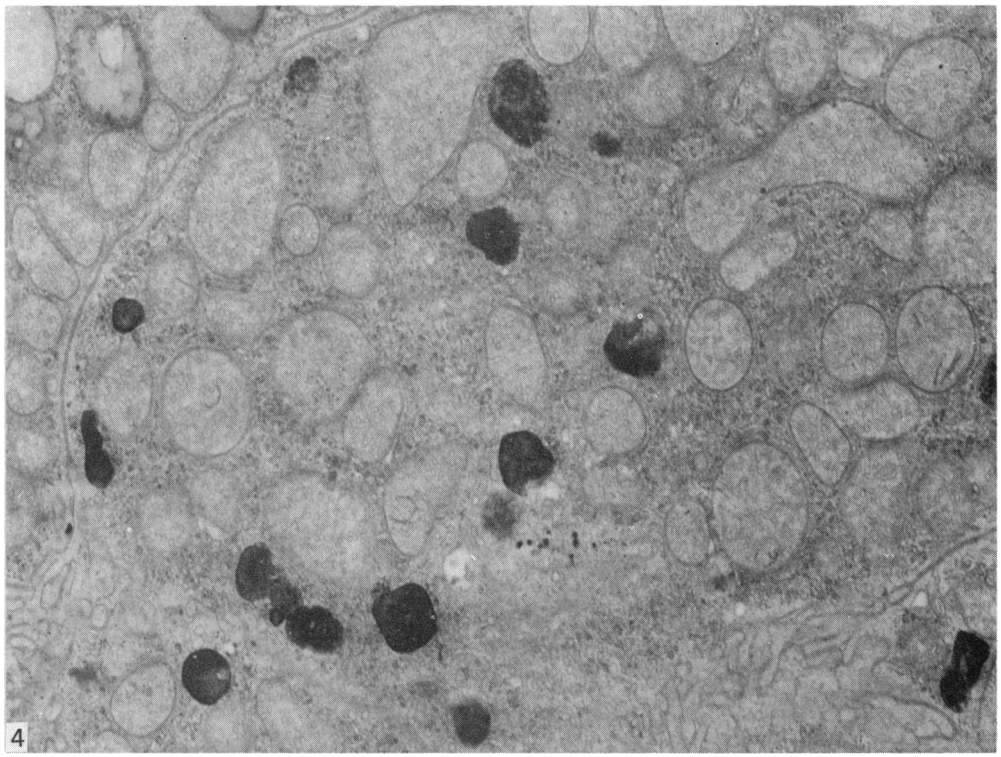

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suggested that the changes in the surface areas of mitochondrial cristae and smooth endoplasmic reticulum membranes are associated with a corresponding change in the activity of enzymes of steroid synthesis.

On these grounds, it is reasonable to assume that these ultrastructural changes represent the morphological counterpart of the angiotensin-induced stimulation of the growth and steroidogenic capacity of zona fasciculata cells. This contention is also supported by personal preliminary data showing that in rats chronically administered with angiotensin the plasma corticosterone concentration is significantly increased. It must be recalled that an analogous finding has been reported by Kasemsri & Nickerson (1976) in renal-encapsulated hypertensive rats. Our karyometric data, also, are in keeping with this view, since the increase in the nuclear volume was found to be associated with an enhanced cellular function (Palkovitz & Fischer, 1968; Nussdorfer *et al.* 1978).

Our ultrastructural observations may easily explain the significant increase in the volume of the lipid compartment in the animals chronically treated with angiotensin. According to some investigators (Christensen, 1975; Nussdorfer *et al.* 1978), smooth endoplasmic reticulum is also involved in the endogenous synthesis of cholesterol from acetate and glucose. Since cholesterol esters are the most important components of the lipid droplets (Moses, Davis, Rosenthal & Garren, 1969; Sand, Frühling, Penasse & Claude, 1972), it is conceivable that the hypertrophy of smooth endoplasmic reticulum would enable zona fasciculata cells to store, as lipid droplets, increased amounts of corticosterone precursors.

The hypertrophy of the Golgi apparatus is also in agreement with previous investigations (Nussdorfer & Mazzocchi, 1972, 1973). It should be recalled that a large body of data suggests that this organelle may be involved in steroid synthesis and release (Christensen, 1975; Idelman, 1978; Nussdorfer *et al.* 1978).

The apparent increase in the number of dense bodies in angiotensin-treated rat zona fasciculata cells deserves some comment. Several earlier studies have described a conspicuous numerical increase in dense bodies in chronically stimulated adrenocortical cells (Szabò, Stark & Varga, 1967; Szabò, Dzsinich, Ökrös & Stark, 1970; Nussdorfer, Mazzocchi & Rebonato, 1971; Hashida & Yunis, 1972). Recently, we have found an increase in dense bodies in zona glomerulosa cells of renovascular hypertensive rats (Rebuffat *et al.* 1979) and in the light of indirect evidence the hypothesis was advanced that they were true hormone-containing secretory organelles. However, this does not seem to be the case in the present study, inasmuch as dense bodies, which invariably show acid phosphatase activity, appear to be typical lysosomes. The significance of this finding remains to be settled, since there is not yet a reasonable agreement as to the function of lysosomes in steroid-producing cells (Nussdorfer *et al.* 1978).

In conclusion, our morphometric data clearly indicate that the renin-angiotensin system exerts a trophic action on the rat zona fasciculata. In previous investigations (Kasemsri & Nickerson, 1976), it was hypothesized that this effect of angiotensin is attributable to ACTH, whose secretion may be enhanced by the stressful condition

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Fig. 3. Zona fasciculata cells of a rat treated for 9 consecutive days with angiotensin. Numerous dense bodies are scattered in the cytoplasm. *ld*, lipid droplet. ×15000.

Fig. 4. Zona fasciculata cells of a rat after 15 days of continuous angiotensin treatment. Numerous pleomorphic Gomori-positive bodies are present. Unstained section. ×16000.

Table 1. *Effects of angiotensin II on morphometric parameters of rat zona fasciculata*

| Parameters | | Controls (6) | 3 days (6) | 6 days (6) | 9 days (6) | 12 days (6) | 15 days (6) |
|---|---|---|---|---|---|---|---|
| Volume of cells, $\mu m^3$ | *P* | 1779·0 ± 78·1 | 1899·4 ± 81·9<br>NS | 2031·8 ± 85·9<br>< 0·02 | 2235·6 ± 95·7<br>< 0·01 | 2409·4 ± 105·7<br>< 0·01 | 2468·6 ± 110·4<br>< 0·01 |
| Volume of nuclei, $\mu m^3$ | *P* | 142·3 ± 6·4 | 140·4 ± 6·1<br>NS | 151·1 ± 6·8<br>NS | 169·8 ± 7·2<br>< 0·01 | 182·8 ± 8·0<br>< 0·01 | 192·4 ± 8·3<br>< 0·01 |
| Volume of mitochondrial compartment, $\mu m^3$ | *P* | 606·4 ± 28·6 | 640·3 ± 29·2<br>NS | 695·7 ± 29·7<br>< 0·02 | 825·5 ± 35·9<br>< 0·01 | 873·2 ± 36·9<br>< 0·01 | 887·9 ± 38·3<br>< 0·01 |
| Surface of mitochondrial cristae, $\mu m^2$ | *P* | 12142·8 ± 584·1 | 12921·4 ± 554·9<br>NS | 15022·8 ± 662·6<br>< 0·01 | 18491·2 ± 784·2<br>< 0·01 | 19559·6 ± 818·6<br>< 0·01 | 21664·8 ± 922·3<br>< 0·01 |
| Surface of smooth endoplasmic reticulum | *P* | 8595·9 ± 368·3 | 9302·0 ± 388·1<br>NS | 9753·4 ± 431·9<br>< 0·05 | 10445·5 ± 448·8<br>< 0·01 | 11539·4 ± 516·2<br>< 0·01 | 12008·5 ± 531·7<br>< 0·01 |
| Volume of lipid compartment, $\mu m^3$ | *P* | 85·7 ± 4·1 | 104·3 ± 4·6<br>< 0·01 | 113·2 ± 5·1<br>< 0·01 | 126·7 ± 5·4<br>< 0·01 | 132·8 ± 5·6<br>< 0·01 | 142·3 ± 6·2<br>< 0·01 |

Animals were treated as described in the text. The number of animals in each group is indicated in parentheses. Each value represents the group mean ± S.E. The degree of variability in the intra-animal determinations, as compared to the intra-group mean, was found by the analysis of variance to be not significant ($P > 0·6$). *P*, level of significance of the difference from the control group. NS, not significant.

related to the experimental model employed (i.e. renal encapsulation). However, in our study the hypothalamo-hypophyseal-adrenal axis of rats was blocked by the contemporary administration of low doses of dexamethasone and ACTH. Therefore, we suggest that angiotensin II directly stimulates the growth of rat zona fasciculata. The functional implications of this contention require further investigations before full elucidation is possible.

SUMMARY

The effects of a chronic administration of angiotensin II on the zona fasciculata of rats treated with dexamethasone and maintenance doses of ACTH were investigated by morphometric methods applied to electron microscopy. It was found that angiotensin induced a significant increase in the volume of cells, nuclei, mitochondrial and lipid compartments as well as in the surface area of smooth endoplasmic reticulum and mitochondrial cristae. Noticeable also were the hypertrophy of the Golgi apparatus and the increase in the number of acid phosphatase-positive dense bodies. These findings are interpreted as indicating that the renin-angiotensin system is involved in the stimulation of the growth and steroidogenic capacity of the rat zona fasciculata.

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