Long-term effects of ACTH combined with angiotensin II on steroidogenesis and adrenal zona glomerulosa morphology in the rat

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Abstract. To test the hypothesis that the trophic action of angiotensin II on the adrenal zona glomerulosa may allow a sustained stimulation of aldosterone by ACTH by preventing the morphological changes of the zona glomerulosa cells into zona fasciculata-like elements we investigated the effects in rats of a 6-day treatment with ACTH\(\times 1\) (100 µg/kg/day) alone or combined with angiotensin II (300 ng/kg/day) on corticosterone and aldosterone production and adrenal morphology. The responsiveness of both steroids to an acute ACTH dose was also studied on the last day of long-term treatment. Morphologic data showed that prolonged ACTH treatment stimulated the growth of zona glomerulosa cells, though it transformed the tubulo-lamellar cristae of mitochondria into a homogeneous population of vesicles. Angiotensin II furthered the trophic effects of ACTH but prevented the mitochondrial transformation. Despite its ability to conserve the well differentiated aspect of the zona glomerulosa cells, the administration of angiotensin II was unable to prevent the fall in the secretion of aldosterone caused by chronic ACTH treatment and its subsequent unresponsiveness to ACTH stimulation.

Among the mechanisms which have been proposed to explain this phenomenon, the most attractive seems that of a direct effect of ACTH upon the zona glomerulosa cells which induces their transformation into a fasciculata-like form (Hornsby et al. 1974; McDougall et al. 1980; Nussdorfer et al. 1982).

In the present study we sought to give support to the hypothesis that elevated angiotensin II levels, by maintaining a stimulation of the zona glomerulosa, may prevent this transformation and thus allow sustained stimulation of aldosterone by ACTH (Gaillard et al. 1983). For this purpose we studied the effects in the rat of prolonged ACTH treatment on aldosterone and corticosterone production as well as on the ultrastructure of the adrenal cells, and tried to counterbalance these effects by concomitant treatment with angiotensin II. Furthermore, on the last day of the experimental period we tested the ability of the adrenals to respond to an acute stimulation by ACTH.

The transitory stimulation of aldosterone secretion by long-term ACTH administration is a well known phenomenon which has been observed in man and in experimental animals (Newton et al. 1968; Biglieri et al. 1969; Müller 1978; McDougall et al. 1980; Aguilera et al. 1981).

Materials and Methods

Thirty-two male Wistar rats weighing 256 ± 12 g (SD) were housed in individual metabolic cages under controlled lighting conditions and fed a normal diet (Kliba
Na\(^+\): 0.25\%, K\(^+\): 0.8\%). Experiments were started after a 5-day adaptation period. Urine was collected daily, and body weight was recorded. The animals were divided into 4 groups and treated for 6 days with ACTH (Synacthen Depot Ciba 100 µg/kg/day sc) and/or angiotensin II-amide (Hypertensin Ciba, Alzet pump 2001, 300 ng/kg/min ip) or saline as shown in Table 1.

On the 7th day all the animals were injected sc with ACTH (Synacthen CIBA 500 µg/kg) and killed by decapitation 30 min later. Trunk blood was collected in tubes containing EDTA for steroid assays.

Urinary free aldosterone and corticosterone were measured by RIA on the same sample after extraction with methylene chloride and fractionation by chromatography (Shapiro et al. 1972; Guelpa-Decorzant et al. 1979; Chabert et al. 1984). The acid-labile conjugate of aldosterone was measured in group A and C (Guelpa-Decorzant et al. 1979). Plasma aldosterone was measured by RIA after chromatography (Chabert et al. 1984). Plasma corticosterone was measured by the competitive protein binding assay of Leclercq et al. (1969) after testing this procedure \(y\) against a RIA procedure for plasma corticosterone \(x\) (Chabert et al. 1984; \(y = 1.04x + 0.80; r = 0.996\); inter-assay variation 5.7%; intra-assay variation 2.5%).

The in vivo functioning of the osmotic pumps inserted ip was controlled in preliminary experiments. The delivery rate was estimated with a pump filled with \(^{125}\)Iangiotensin II solution. The urinary excretion of radioactivity measured during 6 days was 8298 ± 268 cpm per day (cv: 7%) and the mean flow rate was 0.88 µl/h. The stability of angiotensin II-amide (Hypertensin) during the time of operation of the pump was checked in 2 ways. Five rats were implanted with minipumps. After 6 days of angiotensin II infusion (300 ng/kg/min) their systolic blood pressure was measured; it was significantly higher than in the 4 animals serving as controls (141 ± 16 vs 116 ± 2 mmHg; \(P < 0.05\)). Using a HPLC procedure (Nussberger et al. 1985) the presence of angiotensin II-amide was specifically demonstrated in the blood of the same treated rats. Furthermore, the RIA performed on the angiotensin II (1–8 octapeptide) fraction after HPLC showed a significantly decreased level of the peptide in the treated animals when compared with the controls (8.5 ± 1.6 vs 37.8 ± 6 fmol/ml; \(P < 0.05\)).

The adrenal glands were dissected free of fat and processed for electron microscopy as described by Nussdorfer et al. (1982). Thick sections were made by LKB III ultramicrotomes and examined by light microscopy to select the zona glomerulosa. Thin sections were counterstained with lead hydroxide and examined in a Hitachi HS-9 electron microscope. For morphometric assessments the sampling procedure used was that described by Mazzocchi et al. (1983). The average volume of zona glomerulosa cells and the absolute amount of the various organelles per cell were determined according to Nussdorfer (1970), employing conventional stereological procedures (Weibel 1979).

Statistical analysis for biochemical data was done by comparing the values in the treated animals to those of the controls. The Wilcoxon test or Student's \(t\)-test were used to determine statistical significance taken as \(P < 0.05\). Statistical analysis for morphometric data was done by ANOVA and the multiple range test of Duncan. The results are expressed as mean ± SEM unless otherwise stated.

### Results

Body weight increased steadily in the 4 groups of rats during the 6 experimental days but significantly less in the two ACTH-treated groups. Angiotensin II infusion alone or combined with ACTH had no effect. The total weight increase was 29.5 ± 2.3 g in the controls, 11.9 ± 1.3 g in group B \((P < 0.05)\), 30.4 ± 2.4 g in group C (NS) and 15.8 ± 3.6 g in group D \((P < 0.05)\).
Urinary volume was unchanged by angiotensin II infusion alone; it increased with ACTH and rose further with the combined administration of ACTH and angiotensin II. The average volume was $11.7 \pm 0.5$ ml/day in the controls, $14.6 \pm 0.4$ ml/day in group B ($P < 0.05$), $12.2 \pm 0.5$ ml/day in group C and $20.7 \pm 0.7$ ml/day in group D ($P < 0.05$).

Steroid excretion is shown in Fig. 1. The mean urinary excretion of corticosterone was $258 \pm 7.8$ pmol/day in the control group and was not significantly changed by angiotensin II infusion ($259 \pm 8.4$ pmol/day). ACTH alone or combined with angiotensin induced a 10-fold rise. The mean urinary excretion of aldosterone was $14.9 \pm 0.6$ pmol/day in the controls. In the angiotensin II treated animals it was $16.9 \pm 0.8$ pmol/day, and the daily excretion, although continuously higher, was never statistically different from that of the controls ($0.05 < P < 0.1$). The acid-labile conjugate was measured in group A and in group D. The results were not statistically different, being

![Graph showing effects of ACTH and Angiotensin II on urinary excretion of corticosterone and aldosterone.](image)

**Fig. 1.**
Effects of a 6-day treatment with ACTH (100 µg/kg/day) alone or associated with angiotensin II (300 ng/kg/day) on urinary excretion of free corticosterone and aldosterone (mean ± SEM, n = 8, *P < 0.05).

<table>
<thead>
<tr>
<th>Plasma steroid levels</th>
<th>Experimental groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A (controls)</td>
</tr>
<tr>
<td>Corticosterone, nmol/l</td>
<td>961 ± 95</td>
</tr>
<tr>
<td>Aldosterone, nmol/l</td>
<td>0.960 ± 0.128</td>
</tr>
</tbody>
</table>

*Table 2.*
Plasma steroid levels after acute ACTH stimulation in control rats and in rats pre-treated for 6 days with ACTH alone or associated with angiotensin II.
5.5 ± 0.22 pmol/day and 5.1 ± 0.17 pmol/day, respectively. ACTH alone induced only a transient increase of aldosterone excretion, and on the 5th and 6th days the levels were back to control values. With combined ACTH and angiotensin II administration the increase was slightly larger on the first 2 days than with ACTH alone, but the same fall to control values was observed at the end of the experimental period.

Plasma steroid levels after acute ACTH stimulation are shown in Table 2. The corticosterone levels were similar in the controls and in the group which received angiotensin II alone. They were nearly doubled in the 2 groups chronically treated with ACTH. The aldosterone response was slightly higher in the animals treated with angiotensin II alone than in the controls, but this increase was not statistically significant. The two groups which had received either the long-term ACTH treatment alone or that associated with angiotensin II showed similar aldosterone responses, which were considerably lower than in the controls ($P < 0.05$). Basal plasma levels in 12 rats kept under the same housing and feeding conditions were 246 ± 52.6 pmol/l and 107 ± 2.9 nmol/l for aldosterone and corticosterone, respectively.

Morphology indicated (Table 3) that prolonged ACTH administration provoked a significant rise in the volume of zona glomerulosa cells associated with a notable increase in the volume of the mitochondrial compartment and in the surface area per cell of mitochondrial cristae and smooth endoplasmic reticulum (SER) tubules. Electron micrographs showed that in the large majority of zona glomerulosa cells, the increase in the surface area of the inner mitochondrial membranes was coupled with the transformation of the typical tubular cristae (Fig. 2) into vesicles of about 70 nm in diameter (Fig. 3). Chronic infusion of angiotensin II induced similar though less conspicuous morphometric changes (Table 3). Angiotensin II infusion significantly furthered the effects of ACTH on the zona glomerulosa cells (Table 3), but prevented, in a large number of cells, the

**Table 3.**
Morphometric parameters of zona glomerulosa of rats treated with ACTH, AII and ACTH/AII. Mean ± SD.

<table>
<thead>
<tr>
<th>Group</th>
<th>Volume of cells (µm$^3$)</th>
<th>Volume of mitochondrial compartment (µm$^3$/cell)</th>
<th>Surface of mitochondrial cristae (µm$^2$/cell)</th>
<th>Surface of SER (µm$^2$/cell)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (controls)</td>
<td>433.5 ± 46.6</td>
<td>103.3 ± 11.5</td>
<td>1187.9 ± 128.8</td>
<td>2987.5 ± 346.1</td>
</tr>
<tr>
<td>Group B (ACTH)</td>
<td>756.1 ± 96.3</td>
<td>177.8 ± 24.8</td>
<td>2186.5 ± 243.4</td>
<td>5719.7 ± 602.6</td>
</tr>
<tr>
<td>Group C (AII)</td>
<td>614.6 ± 49.9</td>
<td>125.8 ± 35.7</td>
<td>1484.5 ± 156.3</td>
<td>5058.2 ± 583.4</td>
</tr>
<tr>
<td>Group D (ACTH + AII)</td>
<td>927.0 ± 70.0</td>
<td>244.9 ± 30.5</td>
<td>3006.8 ± 361.2</td>
<td>7048.5 ± 809.1</td>
</tr>
</tbody>
</table>

**Figs. 2–4.**

Fig. 2. Zona glomerulosa cells of a control rat. Mitochondria (M) contain tubulo-lamellar cristae. N, nucleus; LD, lipid droplet; SE, subendothelial space. × 18 000.

Fig. 3. Zona glomerulosa cells of a rat chronically treated with ACTH. Round or ovoid mitochondria (M) display vesicular cristae of about 70 nm in diameter. SE, subendothelial space. × 18 000.

Fig. 4. After combined chronic administration of ACTH and angiotensin II, zona glomerulosa mitochondria (M) appear slightly enlarged and show tubulo-convolute cristae. × 21 000.
vesiculation of mitochondrial cristae. Frequently the tubular cristae assumed an evident tortuous and rather convolute appearance (Fig. 4).

Discussion

We recently suggested (Gaillard et al. 1983) that the transient effect of ACTH on aldosterone secretion depends on the state of activity of the renin-angiotensin system. We hypothesized that the renin-angiotensin system was able to delay or prevent the morphological changes induced in the zona glomerulosa by prolonged ACTH stimulation, consequently allowing a persistent production of aldosterone. In the present study performed in rats we therefore tried to counteract the decrease of aldosterone production observed after prolonged ACTH treatment (Müller 1978; Vazir et al. 1981) by administering high doses of angiotensin II concomitantly with ACTH.

Morphometry indicates, in accordance with previous investigations (Nussdorfer 1980, for review), that chronic administration of both ACTH and angiotensin II is able to enhance the growth of the zona glomerulosa. The hypertrophy of zona glomerulosa cells is mainly due to the increase in the volume of the mitochondrial compartment and to the proliferation of SER, the two organelles in which the enzymes of steroid synthesis are contained (Tamaoki 1973). Some lines of evidence indicate a direct relationship between the surface area per cell of mitochondrial cristae and SER tubules and the activity of some steroidogenic enzymes located in them (Mazzocchi et al. 1983).

ACTH administration alone for 6 days induced a sustained rise in urinary corticosterone excretion and a transient rise in aldosterone as observed in the same animal by Haack et al. (1978) and Guelpa-Decorzant et al. (1979). These findings are in keeping with the present morphological data and with recent evidence showing that prolonged ACTH exposure enhances the activity per zona glomerulosa cell of 11β-hydroxylase and 3β-OHSHD isomerase, but not of 18-hydroxylase (Mazzocchi et al. 1986). Though corticosterone production is largely attributable to stimulation by ACTH of the zona fasciculata, the possibility cannot be disregarded that prolonged treatment with ACTH might provoke a shift in the zona glomerulosa cells from production of aldosterone to production of corticosterone.

The present morphometric findings do not appear in agreement with the hormonal results obtained in the angiotensin II-infused animals. Angiotensin II-amide administered for 6 days, in conditions proven to increase blood pressure and to decrease the endogenous peptide, did not produce any significant changes in the excretion of either steroid. In the rat, the biliary route is the major pathway of aldosterone excretion (Morris et al. 1976). Although urinary free aldosterone represents only a very small fraction of total secretion, its measurement has been shown to allow a good evaluation of adrenal function in several experimental situations: stimulation by ACTH, K+, spironolactone and low-salt diet or suppression by dexamethasone (Hilfenhaus 1977; Kley et al. 1978; Guelpa-Decorzant et al. 1979) and high-salt diet (Guelpa-Decorzant, unpublished results). The fact that prolonged angiotensin II infusion provoked a small and not significant rise in urinary free aldosterone levels is surprising and in sharp contrast with the high levels measured in the rat during salt restriction. The same is true for another urinary metabolite of aldosterone, the acid-labile conjugate, whose levels did not rise in angiotensin II treated animals. One possibility is that the high pharmacological dose of angiotensin II employed has altered the metabolism of aldosterone, favouring the biliary route. These results also suggest that peptides other than angiotensin II may be responsible for the large rise observed in urinary aldosterone levels in the rat on a low salt intake.

Angiotensin II infusion potentiates the morphologically appreciable trophic effects of ACTH on the zona glomerulosa cells but prevents the ACTH-induced vesiculation of mitochondrial cristae. It also transiently potentiates the effects of ACTH; in fact aldosterone excretion is significantly higher during the first 2 days of stimulation than it is with ACTH alone. Nevertheless, these levels decreased on the third day to reach the same low value as in the controls at the end of the experimental period.

Overall, these hormonal data are in agreement with the results obtained in humans after repeated ACTH administration during low sodium intake (Biglieri et al. 1969; Gaillard et al. 1983).

The high plasma corticosterone levels observed in the two chronically ACTH-treated groups after
acute ACTH stimulation indicate potentiation of the adrenal response. On the other hand, in the same groups, the acute response of plasma aldosterone to ACTH was completely abolished, the aldosterone concentration being comparable to unstimulated levels. The continuous treatment with angiotensin II alone had no effect on the acute response to ACTH of plasma aldosterone concentration which was identical to that of the control group. These in vivo results do not parallel the in vitro observation of Enyedi et al. (1985) who showed a significantly reduced response of aldosterone to ACTH in glomerulosa cells previously exposed to angiotensin II. Combined with ACTH, the angiotensin pre-treatment was not able to prevent the loss of aldosterone responsiveness to acute ACTH stimulation. These results are in contrast with those obtained in humans after low sodium intake, where a significant increase in plasma aldosterone levels was observed, suggesting again a possible involvement in the response on low sodium diet of peptides other than angiotensin II (Gaillard et al. 1983; Sen et al. 1981).

In conclusion, despite its ability to prevent some of the morphological changes induced in the zona glomerulosa by prolonged ACTH exposure, concomitant angiotensin II administration is unable either to maintain sustained aldosterone stimulation by ACTH or to restore the response to acute stimulation. These data indicate that great caution must be used in interpreting morphological data as indicative of functional changes, and suggest that the morphology of mitochondrial cristae in adrenocortical cells might be indicative of quantitative rather than qualitative differences in the steroidogenic enzymes contained in them. Therefore, some more subtle transformation of zona glomerulosa cells or some still unknown factors have to be evoked to explain the well established transient response of aldosterone to prolonged ACTH stimulation.

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**References**


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