Inhibition of aldosterone turn-off phenomenon following chronic adrenocorticotropic treatment with in vivo administration of antiglucocorticoid and antioxidants in rats

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Chronic adrenocorticotropic (ACTH) treatment in rats leads to a fall in aldosterone secretion (aldosterone turn-off or “aldosterone escape” phenomenon) with a concomitant rise in corticosterone. To elucidate whether ACTH-induced aldosterone suppression is mediated by steroid type II receptor or related to a free-radical effect by over-synthesized corticosterone, we examined the effects of a glucocorticoid antagonist, RU486, and antioxidants dimethyl sulfoxide (DMSO) and vitamin E, on the aldosterone turn-off phenomenon in rats. Each rat received daily for 5 days a different dose of ACTH-Z (5, 10, 20 or 40 μg/100 g body weight) 1 mg RU486/100 g body weight, 100 μl (1.3 mmol) DMSO/100 g body weight or 2 mg vitamin E/100 g body weight with subcutaneous injection. Plasma steroid levels and in vitro release of steroids from the adrenal capsule were measured. The ACTH-Z treatment caused a dose-dependent increase in corticosterone and a decrease in aldosterone in both plasma and adrenal capsule experiments, as well as an increase in adrenal weights. For the following study 5 μg/100 g body weight of ACTH-Z was used. Administration of RU486 alone caused no change in plasma aldosterone level compared to controls, even though the steroid type II receptor was blocked, as evidenced by significant increases in plasma ACTH and corticosterone levels. Concomitant administration of RU486 and ACTH-Z increased both plasma corticosterone and aldosterone levels (p< 0.01) but decreased adrenal capsule corticosterone production (p< 0.05) compared to the rats treated with ACTH-Z alone. Treatment with DMSO alone caused a significant increase in plasma ACTH and corticosterone level (p< 0.05) but no change in plasma aldosterone level or adrenal capsule corticosterone and aldosterone production. The ACTH-induced aldosterone decrease was completely prevented by DMSO administration in both plasma and adrenal capsule experiments (p< 0.01). Vitamin E administration resulted in the elevation of plasma levels of ACTH and corticosterone (p< 0.01 and < 0.05) but not aldosterone, and it also increased adrenal capsule corticosterone production (p< 0.01) but not aldosterone production. By vitamin E administration, the ACTH-induced aldosterone decrease was suppressed almost completely in plasma (p< 0.01) and partially in adrenal capsule experiments (p< 0.01) compared to rats treated with ACTH-Z alone. Our findings suggest that RU486, DMSO and vitamin E inhibit the ACTH-induced aldosterone turn-off phenomenon in plasma, possibly due to the increase in activity of P-450aldo through antioxidant action or a steroid type II receptor blocking action.

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It is generally known that pituitary adrenocorticotropic (ACTH) is a potent stimulator of corticosterone and aldosterone secretion in rat, but ACTH plays a specific role because its acute and chronic effects differ completely with respect to aldosterone secretion. Acute ACTH treatment provokes a rise in the plasma concentration of both corticosterone and aldosterone, while chronic ACTH treatment leads to a dramatic fall in aldosterone production concomitantly with an increase in corticosterone production (aldosterone turn-off or “aldosterone escape” phenomenon) (1–7).

A number of suggestions have been made as to the possible mechanism of the effect of chronic ACTH on aldosterone biosynthesis. One of these involves the direct effect of ACTH on the adrenal zona glomerulosa cell, which leads to hypertrophy of adrenal gland by increasing adrenocortical cell volume and number, and functional and morphological transformation of zona glomerulosa cells into either intermediate or fasciculata-like cells (1, 3, 6, 8–11). The other involves inhibition of the late steps of aldosterone biosynthesis by ACTH through an inappropriate increase in adrenal products, such as deoxycorticosterone (DOC) and corticosterone, which suppress the subsequent conversion of DOC to...
aldosterone and 18-hydroxycorticosterone (18-OH-corticosterone) through suppression of the renin-angiotensin system and potassium depletion (1. 2, 11–14). However, a study that maintained potassium balance and stimulated the renin-angiotensin system still showed a fall in the aldosterone level after chronic ACTH treatment (15). On the other hand, it is well known that high concentrations of some steroids in vitro can lead to a decreased synthesis of the end-product steroids through the inactivation of some of the cytochrome P-450 due to a free-radical mechanism (16). In fact, aldosterone synthesis in cultured bovine (16, 17) and rat (18) adrenocortical zona glomerulosa was suppressed by cortisol, which interacts with glomerulosa cytochrome P-450<sub>aldo</sub>, and suppression was prevented by the addition of antioxidants (16, 19). Mooradian (20) recently showed the antioxidant properties of corticosterone and cortisone using a phycocerythrin fluorescence-based assay for peroxyl radicals. The enzyme catalyzing the steps in the synthesis of aldosterone in rats and humans is P-450<sub>aldo</sub> (P-450<sub>11β</sub>), the amino acid sequence of which in rats has 81–83% homology with P-450<sub>11β</sub> (21, 22). These results suggest that induction of P-450<sub>aldo</sub>, like P-450<sub>11β</sub>, may be affected by the free-radical effects of corticosterone in rats.

In view of these considerations, the investigators investigated whether suppression of aldosterone secretion during chronic ACTH administration is due to the free-radical effects of over-synthesized corticosterone or mediated by steroid type II receptor in the rat. To this end, the chronic effect of ACTH treatment of aldosterone secretion in the rat was investigated with and without glucocorticoid receptor antagonist 17β-hydroxy-11β-(4-dimethylaminophenyl)-17α-(1-propynyl)estra-4,9-dien-3-one (RU486), or the antioxidants dimethyl sulfoxide (DMSO) and vitamin E.

Materials and methods

Chemicals

The following materials were purchased: corticosterone<sup>125</sup>I RIA kit from ICN Biomedicals Customer Service Department, Inc. (Costa Mesa, CA), aldosterone RIA kit from Shionogi Pharmaceutical Co. (Osaka, Japan), ACTH IRMA kit from Mitsubishi Petrochemical Co. (Tokyo, Japan), DMSO (highest purity 99.8%) from Sigma Chemical Co. (St Louis, MO), vitamin E (tocopherol acetate) from Elsai Co. (Tokyo, Japan), [1,2,3]<sup>H</sup> 18-OH-corticosterone and a liquid scintillator, ACS II, from Amersham Co. (Arlington Heights, Ill.). The 18-OH-corticosterone antiserum was kindly supplied by Dr Celso E Gomez-Sanchez (University of Missouri-Columbia, MO, U.S.A.). RU486 and cortrosyn Z (ACTH-Z) were kindly supplied from Roussel-Uclaf (Romainville, France) and Daiichi Pharmaceutical Co. (Tokyo, Japan), respectively.

Treatment of animals

Adult male Wistar rats weighing about 200 g each were obtained from SLC Inc. (Nagoya, Japan). Food and water were freely available. The rats were housed under controlled temperature (22–28°C) and lighting conditions, on days consisting of 14 h of light (06.00–20.00 h) and 10 h of darkness (20.00–06.00 h). The rats were divided into seven groups (N = 5), and each group was treated daily for 5 days with ACTH-Z, RU486, DMSO, vitamin E. As vehicle, sesame oil (N = 10) or saline (N = 10) was injected for 5 days. Because the hormonal data were not different between groups with treatments (data not shown), we chose the data of the rats injected with saline as control. In all of our experiments, rats were treated at 08.30 h and were killed between 16.00 h and 17.00 h (8 h after the last injection of ACTH-Z) to perform the experiments under as high a concentration of corticosterone as possible. The ACTH-Z was injected subcutaneously at doses of 5, 10, 20, or 40 µg/100 g body weight. RU486 was dissolved in sesame oil and injected subcutaneously at a dose of 1 mg/100 g body weight. Dimethyl sulfoxide at a dose of 50 µl (0.65 mmol)/100 g body weight was given subcutaneously twice a day. Vitamin E was injected subcutaneously at a dose of 2 mg/100 g body weight.

Adrenal weights increased dose dependently with ACTH-Z treatment for 5 days (data not shown). There was no significant difference in the body weight between the groups receiving RU486, vitamin E and DMSO with or without 5 µg/100 g body weight per day of ACTH-Z for 5 days (Table 1), while the adrenal weight was significantly increased in the groups treated with ACTH-Z.

Tissue preparation and incubation

The rats were killed by decapitation and the trunk blood was collected in a prechilled tube containing EDTA. Plasma was obtained and stored at −70°C until hormone measurements. The adrenal glands were

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weight (mg)</th>
<th>Adrenal weight (mg/pair)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>244.0 ± 3.1</td>
<td>44.3 ± 1.1</td>
</tr>
<tr>
<td>RU486</td>
<td>237.4 ± 2.4</td>
<td>45.5 ± 1.7</td>
</tr>
<tr>
<td>DMSO</td>
<td>245.3 ± 2.2</td>
<td>47.8 ± 1.4</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>239.9 ± 2.4</td>
<td>44.3 ± 0.7</td>
</tr>
<tr>
<td>ACTH-Z</td>
<td>236.4 ± 2.2</td>
<td>68.9 ± 2.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ACTH-Z + RU486</td>
<td>238.6 ± 2.5</td>
<td>69.6 ± 3.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>ACTH-Z + DMSO</td>
<td>237.0 ± 2.0</td>
<td>75.6 ± 1.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ACTH-Z + vitamin E</td>
<td>235.1 ± 2.6</td>
<td>68.9 ± 1.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>p < 0.01 vs vehicle.<br><sup>b</sup>p < 0.01 vs without ACTH-Z.
quickly removed, cleaned, weighed and placed in Krebs-Ringer phosphate buffer solution (127.5 mmol/l NaCl, 5.1 mmol/l KCl, 12.5 mmol/l NaH₂PO₄, 1.3 mmol/l MgSO₄·7H₂O, 1.4 mmol/l CaCl₂, 11.1 mmol/l glucose, 0.2% BSA, pH 7.4) on ice for brief periods before separation into capsule and inner zones by gentle compressing between clear glass plates (3). The experiment on steroid production was performed as described previously by Vazir et al. (3) and Abaysekala et al. (6). Briefly, the tissue derived from one pair of the adrenals was preincubated for 1 h in 2 ml of medium at 37°C under conditions of 95% O₂ and 5% CO₂ in a shaking water-bath to stabilize the tissues. After changing the medium for a fresh one, the incubation was continued for a further 2 h under the same conditions, and the media were frozen at −70°C until steroid measurements were performed.

**Hormone measurement**

Plasma and media aldosterone concentrations were measured with an aldosterone RIA kit following the appropriate dilution with aldosterone-free serum. Cross-reactions of antiserum against other corticosteroids were as follows: 0.00016% for corticosterone, 0.000026% for progesterone, 0.015% for 18-OH-corticosterone and 0.00012% for DOC. Plasma and media corticosterone concentration were measured with a corticosterone 125I RIA kit. Cross-reactivity of anti-corticosterone antibody was 0.34% for DOC and 0.03% for aldosterone. 18-Hydroxycorticosterone was measured by RIA after extracting with dichloromethane as reported previously (23–25). Plasma ACTH concentration was determined by immunoradiometric assay using an ACTH IRMA kit.

**Analysis of data**

Experimental data are presented as means±SEM. Statistical analysis was performed using a non-parametric Mann–Whitney U test. Differences were considered to be significant when p<0.05.

**Results**

**Effect of ACTH-Z**

Although plasma corticosterone increased in a dose-dependent manner with various doses (5–40 µg/100 g body weight per day) of ACTH-Z administered for 5 days, plasma aldosterone was even decreased with 5 µg/100 g body weight per day (Table 2). Plasma aldosterone was further decreased with 20 and 40 µg/100 g body weight per day of ACTH-Z for 5 days. Similar effects of ACTH-Z treatment were observed on corticosterone, aldosterone and 18-OH-corticosterone production from the adrenal capsule (Table 2). These decreases in plasma level and adrenal capsule production of aldosterone were not observed with 2 days of treatment with 5 µg/100 g body weight per day of ACTH-Z (Table 3). Based on these results, 5 µg/100 g body weight per day of ACTH-Z for 5 days was chosen as the experimental condition in the following experiments for the effects of RU486, DMSO and vitamin E.

**Effect of RU486**

Administration of 1 mg RU486/100 g body weight per day for 5 days resulted in significant increases in plasma ACTH and corticosterone levels as compared to without RU486 (ACTH, 28.85 ± 4.81 vs 16.42 ± 1.93 pmol/l, p < 0.05; corticosterone, 0.81 ± 0.01 vs 0.53 ± 0.08 µmol/l, p < 0.05) (Fig. 1.) Mean levels of plasma aldosterone were also increased; but were not significantly different (Fig. 1). Concomitant administration of 5 µg/100 g body weight per day for ACTH-Z and 1 mg/100 g body weight per day of RU486 resulted in a fourfold rise in plasma aldosterone (ACTH-Z alone, 1.51 ± 0.25 vs ACTH-Z plus RU486, 5.59 ± 0.33 nmol/l; p < 0.01) as well corticosterone (ACTH-Z alone, 0.87 ± 0.09 vs ACTH-Z plus RU486, 3.82 ± 0.59 µmol/l; p < 0.01) as compared with rats treated with ACTH-Z alone (Fig. 1). However, different from the plasma results, RU486 administration did not affect adrenal capsule corticosterone and aldosterone production (Fig. 2). Concomitant administration of 1 mg of RU486 and 5 µg of ACTH-Z per 100 g body weight per day for 5 days significantly decreased adrenal capsule corticosterone production (6.40 ± 0.61 vs 14.41 ± 1.81 pmol.l⁻¹mg⁻¹ tissue; p < 0.01), but not aldosterone production, as compared to administration of ACTH-Z alone (Fig. 2).

**Effects of DMSO and vitamin E**

Treatment with DMSO for 5 days significantly increased plasma levels of ACTH and corticosterone (ACTH, 28.14 ± 3.76 vs 16.41 ± 1.92 pmol/l, p < 0.01; corticosterone, 1.81 ± 0.08 vs 0.53 ± 0.07 µmol/l, p < 0.05), and vitamin E treatment caused similar changes in plasma levels of ACTH and corticosterone to DMSO (ACTH, 32.11 ± 0.86 vs 16.41 ± 1.92 pmol/l, p < 0.01; corticosterone, 1.06 ± 0.15 vs 0.53 ± 0.07 µmol/l, p < 0.05) (Fig. 1). However, neither DMSO nor vitamin E affected the plasma aldosterone level (Fig. 1). Concomitant administration of ACTH-Z and DMSO or vitamin E caused an increased response of plasma corticosterone as compared with rats treated with ACTH-Z alone. Moreover, both DMSO and vitamin E almost completely suppressed the inhibitory response of plasma aldosterone induced by 5 days of ACTH-Z treatment (without ACTH, 5.01 ± 0.79; with ACTH-Z alone, 1.51 ± 0.25; ACTH-Z plus DMSO, 5.01 ± 0.53; ACTH-Z plus vitamin E, 5.47 ± 0.62 nmol/l; p < 0.01) (Fig. 1). In other words, the aldosterone response to ACTH-Z plus DMSO or ACTH-Z plus vitamin E was
Table 2. Effect of 5, 10, 20 and 40 µg ACTH-Z/100 g body weight per day for 5 days on corticosterone (B), aldosterone (Aldo) and 18-OH-corticosterone (18-OH-B) in plasma and adrenal capsule experiments.

<table>
<thead>
<tr>
<th>ACTH-Z dose (µg/100 g body wt)</th>
<th>Plasma</th>
<th>Adrenal capsule (pmol.h⁻¹.mg⁻¹ tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B (µmol/l)</td>
<td>Aldo (nmol/l)</td>
</tr>
<tr>
<td>0 (N = 10)</td>
<td>0.33 ± 0.07</td>
<td>5.01 ± 0.78</td>
</tr>
<tr>
<td>5 (N = 5)</td>
<td>0.87 ± 0.08*</td>
<td>1.51 ± 0.25**</td>
</tr>
<tr>
<td>10 (N = 5)</td>
<td>1.32 ± 0.15</td>
<td>1.23 ± 0.04**</td>
</tr>
<tr>
<td>20 (N = 5)</td>
<td>2.07 ± 0.27*</td>
<td>0.72 ± 0.01**</td>
</tr>
<tr>
<td>40 (N = 5)</td>
<td>2.01 ± 0.07*</td>
<td>0.68 ± 0.07**</td>
</tr>
</tbody>
</table>

*p < 0.05 and **p < 0.01 vs without ACTH-Z

almost equal to the response in the control group without ACTH-Z or even somewhat increased as compared to 1 and 2 days of ACTH-Z treatment (Table 3 and Fig. 1). There was no significant difference between the aldosterone response to ACTH-Z plus DMSO and that to DMSO alone, or ACTH-Z plus vitamin E and that to vitamin E alone (Fig. 1).

In the adrenal capsule experiments, treatment with DMSO alone did not increase corticosterone and aldosterone production. When DMSO was administered with ACTH-Z for 5 days, aldosterone production inhibited by ACTH was increased significantly (0.57 ± 0.04 vs 0.19 ± 0.01 pmol.h⁻¹.mg⁻¹ tissue; p < 0.01) and was almost equal to the normal control level (Fig. 2). On the other hand, vitamin E by itself had no effects on aldosterone production but it increased corticosterone production. Concomitant administration

Fig. 1. Plasma ACTH (a), corticosterone (b) and aldosterone (c) levels in rats treated daily for 5 days with saline, 5 µg ACTH-Z/100 g body weight, 1 mg RU486/100 g body weight, 100 µl (1.3 mmol) DMSO/100 g body weight, and 2 mg vitamin E/vitamin E/100 g body weight, or ACTH-Z plus RU486, DMSO and vitamin E. All data are means ± SEM (saline, N = 10; others, N = 5). Closed and hatched bars represent without or with ACTH-Z, respectively: *p < 0.05 and **p < 0.01 vs normal controls (saline), p < 0.05 and p < 0.01 vs ACTH-Z alone.

Fig. 2. Adrenal capsule corticosterone (a) and aldosterone (b) production in rats treated daily for 5 days with saline, 5 µg ACTH-Z/100 g body weight, 1 mg RU486/100 g body weight, 100 µl (1.3 mmol) DMSO/100 g body weight and 2 mg vitamin E (vit E)/100 g body weight, or ACTH-Z plus RU486, DMSO and vitamin E. All data are means ± SEM (saline, N = 10; others, N = 5). Closed and hatched bars represent without or with ACTH-Z, respectively. *p < 0.05 and **p < 0.01 vs normal controls (saline), p < 0.05 and p < 0.01 vs ACTH-Z alone.
Treatment by days even in in
Our Discussion that Z higher
adrenal
plasma and inhibited by vitamin E also relieved the ACTH-induced "aldosterone escape" phenomenon in the adrenal capsule experiment.

Discussion
Our present findings showed that 5-day chronic ACTH-Z treatment in rats provoked a dose-dependent decrease in aldosterone and an increase in corticosterone in plasma and adrenal capsule experiments, accompanied by an increase in the adrenal weight. The adrenal capsule 18-OH-corticosterone production also decreased with increasing ACTH doses (Table 2). These findings confirmed previous results and indicated that even 5 µg ACTH-Z /100 g body weight per day for 5 days could induce an aldosterone turn-off phenomenon.

Plasma ACTH and corticosterone levels increased with treatment of RU486, DMSO or vitamin E. Moreover, such treatments combined with ACTH also resulted in potentiation of the plasma corticosterone response compared to that in ACTH alone. Because RU486 prevents the biological effects of glucocorticoid by competing with these hormones binding to the intracellular glucocorticoid receptor, administration of RU486 blocks negative feedback, resulting in increased plasma levels of ACTH and cortisol (26), which was consistent with our present findings. In contrast, the results of the adrenal capsule experiments showed no increase in corticosterone synthesis in RU486 treated rats. This discrepancy would be due to inhibition of steroidalogenic enzyme activities, including 11-hydroxylase and 21-hydroxylase, by RU486 in rat adrenal, as shown by Albertson et al. (26). They similarly observed that adrenal steroidalogenic enzyme activities were inhibited by RU486 treatment for 7 days in hypophysectomized—castrated—ACTH-replaced rats in spite of a lack of effect on plasma corticosterone levels. As they postulated, the longer administration period might be necessary to provide sufficient adrenal suppression for blood corticosterone levels to reflect the effect of RU486 on steroidalogenic enzymes.

Although DMSO or vitamin E, with or without ACTH-Z, caused the same responses in plasma ACTH or corticosterone as RU486, vitamin E also increased corticosterone synthesis in adrenal capsule experiments (Fig 2). There have been few studies on the effects of antioxidants on glucocorticoid synthesis. Serum corticosterone and adrenal corticosterone content obtained from vitamin E-sufficient rats were increased significantly compared to those from vitamin E-deficient rats (27). Furthermore, inhibition of ACTH-induced corticosterone synthesis in vitamin E-deficient rats by ascorbate was reversed by vitamin E supplementation (28). On the other hand, silibinin, an antioxidant, slightly decreased the basal secretion of cortisol in human pathological adrenocortical cells but potentiated the ACTH-induced cortisol response (29). These studies suggest that increases in corticosterone secretion and synthesis by in vivo administration of DMSO and vitamin E may be attributed to their antioxidant properties. To the best of our knowledge, there have been no reports on the effects of both substances or other antioxidants on ACTH secretion or ACTH metabolism. The reason why negative feedback mechanism did not operate in in vivo DMSO and vitamin E treatments has yet to be elucidated.

RU486, DMSO or vitamin E administration did not affect the basal plasma aldosterone level, but all three drugs prevented ACTH-induced inhibition of plasma aldosterone. In the adrenal capsule experiments, however, aldosterone production was not affected by RU486 and, also, the ACTH-induced aldosterone turn-off phenomenon was not inhibited with it. Although DMSO or vitamin E alone did not increase basal aldosterone production, both of them prevented the decrease in aldosterone production by chronic ACTH treatment. The effect of DMSO on aldosterone synthesis might be more potent than that of vitamin E and in fact prevented the ACTH-induced aldosterone decrease almost completely. Concerning the discrepancy of plasma aldosterone levels and its production in the
adrenal capsule experiments by RU486, the period of RU486 administration could be significant, as mentioned in the above discussion. Another explanation is that the turnover of steroid(s) in the adrenals may be affected by the RU486 treatment. A recent study indicated that RU486 had potent antioxidant properties (30), and the observed finding in the present study is a reflection of the complex action of RU486. Mean aldosterone production by ACTH treatment for 1 and 2 days was 0.76 pmol.h⁻¹mg⁻¹ tissue, and this was decreased to 0.19 pmol.h⁻¹mg⁻¹ tissue (about 74% decrease) with 5 days of ACTH treatment (Table 3 and Fig. 2). Thus, vitamin E, although partially, recovered aldosterone production from 0.19 to 0.28 pmol.h⁻¹mg⁻¹ tissue, which still represented about a 63% decrease compared to that during the short-term ACTH treatment. Of interest, although only DMSO prevented the adrenal capsule aldosterone decrease completely, RU486, DMSO and vitamin E almost completely inhibited aldosterone suppression by chronic ACTH treatment in plasma. RU486 and vitamin E may interfere with the in vivo metabolism of aldosterone, e.g. in the liver, which might participate in prolonging the half-life of the steroid in plasma. Many studies have been performed to prevent the aldosterone turn-off phenomenon induced by chronic administration of ACTH. However, such attempts have failed. Only pulsatile infusion of ACTH in humans maintained aldosterone secretion over time (31). Hence, the present findings are very important for understanding the physiological relationship between intra-adrenal corticosterone and aldosterone production during chronic ACTH treatment.

In culture rat and bovine adrenocortical cells, cytochrome corticosterone methyl oxidase (here termed P-450 ald) appears to be subject to inactivation by steroids such as cortisol and corticosterone at concentrations likely to exist in the adrenal cortex in vivo, causing loss of P-450 ald activity and loss of aldosterone and 18-OH-corticosterone production (17, 18). Such loss of aldosterone production was prevented by antioxidants such as DMSO and metyrapone (17, 32). On the other hand, treatment of rats with ACTH for 4 days completely suppressed the expression of P-450 ald (CYP11B2) mRNA in the glomerulosa cell (33). Thus, long-term ACTH treatment appears to inhibit aldosterone synthesis by blocking its synthesis rather than by promoting its oxidative destruction. These findings suggest that over-synthesized corticosterone by chronic ACTH stimulation may act as a pseudosubstrate for decreased P-450 ald, stimulate the release of oxygen radicals and cause inhibition of lipid peroxidation and loss of enzyme activity (34) or direct inhibition of gene expression of the enzyme. Furthermore, in a case of non-salt-losing congenital adrenal hyperplasia with a low level of plasma cortisol and an elevated level of plasma aldosterone, 3 days of ACTH administration did not turn off aldosterone secretion (35). This might also indicate that the phenomenon was due to the action of excessive glucocorticoids. However, the effects of in vivo administration of DMSO or vitamin E on ACTH or steroidogenesis have not yet been investigated. The present study indicated that in vivo administration of DMSO, vitamin E and RU486 relieved the ACTH-induced aldosterone turn-off phenomenon. Dimethyl sulfoxide and vitamin E, as antioxidants, would protect the P-450 ald from the injury induced by the prooxidant properties of high concentrations of corticosterone or potentiate the residual activities of the enzyme. Furthermore, RU486 could inhibit the antioxidant action of over-secreted corticosterone as an antiglucocorticoid. In the present study, we could not distinguish the antioxidant- and antiglucocorticoid-induced effects on aldosterone synthase production or activity. In depth analysis of the mechanism, including the effects on steroidogenic enzyme induction at the mRNA level, will be needed for further elucidation.

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