Ascorbate depletion prevents aldosterone stimulation by sodium deficiency in the guinea pig

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The concentration of ascorbic acid (vitamin C) in the adrenal cortex is higher than in any other organ. The role of vitamin C in the adrenal cortex is unknown. But data obtained with bovine adrenocortical cells in vitro favour its role as an antioxidant that especially protects aldosterone synthesis from damaging lipid peroxides. Alternatively, vitamin C could act as part of an auxiliary electron transport system for the last step of aldosterone synthesis. The effects of vitamin C depletion on adrenocortical function cannot be studied in the human for ethical reasons, so we subjected different groups of guinea pigs to vitamin C depletion, sodium depletion and combined vitamin C and sodium depletion. Other groups of animals on normal or vitamin C-deficient diets received high-dose adrenocorticofrophin (ACTH) injections for 3 days before sacrifice. Fifteen days of a vitamin C-free diet led to very low vitamin C levels in adrenals, liver and plasma without clear signs of scurvy. At this time, plasma aldosterone and aldosterone secretion by isolated adrenal cells were stimulated significantly by sodium deficiency. Simultaneous vitamin C depletion completely abolished the rise in aldosterone in vivo and in vitro, significantly reduced the conversion of [3H]deoxycorticosterone to [3H]aldosterone and impaired renal sodium conservation. Plasma renin activity (PRA), plasma ACTH and serum potassium were not different in the sodium-depleted and sodium plus vitamin C-depleted groups. Sodium depletion did not affect cortisol. Vitamin C depletion led to a significant increase in plasma cortisol without an increase in ACTH, while in vitro secretion of cortisol was slightly decreased. These findings seem to be due to decreased hepatic cortisol metabolism. Three days of ACTH treatment led to a large increase in plasma cortisol and in vitro secretion, while plasma aldosterone and in vitro aldosterone secretion (and PRA) were greatly suppressed. This effect of ACTH was not changed by vitamin C depletion. In conclusion, our studies have demonstrated for the first time a permissive role of vitamin C in the adaptation of aldosterone secretion and of sodium excretion to sodium deficiency, which is an important physiological function of aldosterone. The molecular mechanisms by which vitamin C is involved in aldosterone synthesis await further studies.

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Ascorbate is concentrated in the adrenal gland to very high levels compared with other organs (1). The first isolation of vitamin C was accomplished by the extraction of bovine adrenal cortex by Szent-Györgyi in 1927 (2). Adrenal cells are equipped with a specific uptake mechanism for vitamin C (3), and with mechanisms for the regeneration of vitamin C from monodehydroascorbate and dehydroascorbate (4). The involvement of vitamin C in steroidogenesis has been suggested for a long time. Although the earliest assays for the estimation of ACTH are based on the observation that the stimulation of steroid synthesis by ACTH is accompanied by vitamin C depletion of the adrenal (5), the role of ascorbate in the adrenal cortex is unknown. Two different in vitro findings point to the importance of vitamin C for the function of cytochrome P-450 enzymes in aldosterone synthesis:

(i) Hornsby and Crivello (6, 7) showed that the capacity of bovine adrenocortical cells in primary culture to synthesize aldosterone rapidly decreased within 1 or 2 days, but this process could be halted by ascorbic acid or other antioxidants (8). These authors hypothesized that abundant steroids in cell culture, like cortisol or androstenedione, form pseudosubstrate complexes with the cytochrome P-450 enzyme 11–hydroxylase (which catalyses cortisol and aldosterone synthesis in the bovine adrenal). The complex accepts an electron and a molecule of oxygen but, instead of substrate oxidation, a superoxide (O2−) molecule is formed that damages the enzyme, possibly via the formation of lipid peroxides (9). This process seems to be antagonized by ascorbic acid (10). Vitamin C could also protect cytochrome P-450 enzymes by regenerating another antioxidant, vitamin E, from αtocopheroxyl radical (11, 12).

(ii) Hall, Yanagibashi and Kobayashi (13) measured steroid synthesis in mitochondria from beef zona
glomerulosa and zona fasciculata cells. In contrast to the human and the rat (14, 15), whose zona glomerulosa expresses a specific aldosterone synthetase, the 11-hydroxylase catalyses both cortisol synthesis in the bovine fasciculata and aldosterone synthesis in the glomerulosa (13). Conditions in the guinea pig are unknown. It appears that high activities of semidihydroascorbate reductase and NADH-cytochrome C reductase in bovine zona glomerulosa mitochondria provide an auxiliary electron transport system that enables these cells to form aldosterone, while the 11-hydroxylase of the zona fasciculata is restricted to the formation of corticosterone and 18-OH-corticosterone.

Both hypotheses assigning vitamin C a role in aldosterone synthesis suggest that ascorbate depletion of the adrenal would lead to an impairment of aldosterone synthesis. To test this hypothesis based on in vitro experiments, we investigated for the first time the influence of in vivo vitamin C deficiency on aldosterone synthesis in guinea pigs, a species that needs ascorbate as a vitamin like the human. Aldosterone secretion was stimulated by a low sodium diet. Because scurvy stimulates cortisol secretion, probably through ACTH (16), which also influences aldosterone secretion, we attempted to study aldosterone secretion in a state of vitamin C depletion before scurvy develops. To compensate for a potential rise in plasma ACTH by developing scurvy, subgroups of vitamin C-repleted and -depleted guinea pigs were treated with ACTH to elevate plasma ACTH to the same supraphysiological level.

Materials and methods

**Materials**

Adrenocorticotrophin (1–24) (Synacthen® Depot 100 IU/ml) was obtained from Ciba-Geigy (Basel, Switzerland), 10% ketamine from Sanofi Ceva (Düsseldorf, Germany) and xylazine (Rompun®; 2%) from Bayer (Leverkusen, Germany). Sterofundin® solution for electrolyte replacement was purchased from Braun (Melsungen, Germany). Gentamicin solution for injection from Ratiopharm (Ulm, Germany) and nystatin as a sterile powder from Lederle (Wolfstratshausen, Germany) were used in vitro. l-Ascorbic acid, d-α-tocopherol, d-α-tocopheryl acetate and steroids were purchased from Sigma (Munich, Germany). Methanol, n-hexane and 2-propanol were obtained from Merck (Darmstadt, Germany) as LiChrosolv® quality. To characterize the immunoassay antibody, guinea pig ACTH was bought from Auspep (Parkville, Australia). Anti-cortisol-3-(CMO)HSA and anti-aldosterone-3-(CMO)BSA antibodies and Sepapore® tablets with dextrancoated charcoal for radioimmunoassays were purchased from Steranti (St Albans, UK). Radioactive steroids were obtained from Amersham Büchler (Braunschweig, Germany): [1,2,6,7-3H]cortisol (2.33 TBq/mmol) and [1,2,6,7-3H]aldosterone (2.66 TBq/mmol). Tritium-labelled steroids were purified by HPLC before use. Sep-Pak® C18 cartridges were obtained from Waters/ Millipore (Milford, USA); HPLC columns (250 × 4 mm) and precolumns (5 × 4 mm) packed with Spherisorb S5 ODS II 5 µm or LiChrosorb Diol 5 µm were bought from Kruauer (Berlin, Germany). A radioactivity monitor LB 506 C1 from Berthold (Wildbad, Germany) was used.

Outbred male Duncan Hartley guinea pigs (280–320 g) were provided by Moellegaard Breeding Centre (Schönwalde, Germany). Semipurified experimental diets for guinea pigs were obtained from Ssniff (Soest, Germany).

**In vivo experiments**

The guinea pigs were kept under standard conditions (controlled temperature at 20° ± 2°C and 40–60% humidity, 12-h light period from 6.00 to 18.00 h, food and tap water ad libitum). Within 4 weeks they were adapted carefully to the control diet (1500 mg/kg vitamin C, 120 mg/kg vitamin E, 2000 mg/kg sodium, 6500 mg/kg potassium, 3200 mg/kg chloride) and to single housing in metabolic cages. The daily food intake varied between 15 and 30 g.

As the experiment started, the animals were divided randomly into six groups (n = 7–9): (A) controls; (B) vitamin C-depleted; (C) ACTH-treated; (D) ACTH-treated and vitamin C-depleted; (E) sodium-depleted; (F) sodium-depleted and vitamin C-depleted.

The feeding periods lasted 15 days, which is the time required to deplete the adrenal glands of vitamin C without causing visible symptoms of scurvy, as we found out in a pilot study. The animals were weighed every day and urine was collected every 24 h. The vitamin C-depleted diets (groups B, D and F) contained <10 mg/kg ascorbate and the vitamin C-repleted diets (groups A, C and E) contained 1500 mg/kg ascorbate. The sodium-depleted diets (groups E and F) contained 200 mg/kg sodium and 1800 mg/kg chloride and the sodium-repleted diets (groups A, B, C and E) contained 2000 mg/kg sodium and 3200 mg/kg chloride. Stimulation with ACTH (groups C and D) was carried out by injecting 10 IU of depot ACTH subcutaneously twice a day (at 7.00 h and 18.00 h) for the last 3 days (days 13–15). All other animals were injected with 0.1 ml of 0.9% saline as a placebo in the same manner. About 2½ h after the last injection of ACTH or saline (on the morning of day 16), the animals were anaesthetized with 100 mg/kg body weight ketamine combined with 4 mg/kg xylazine by intramuscular injection. Blood samples were obtained by heart puncture with a vacutainer system (Becton and Dickinson, Heidelberg, Germany). The animals were then killed by a stroke on the neck, and adrenals and the liver were removed. Plasma treated with EDTA for the analysis of ACTH, aldosterone, cortisol and PRA was stored at –80°C and
serum at $-180^\circ$C until analysis of vitamin C, vitamin E, sodium and potassium. Liver was frozen at $-180^\circ$C immediately after removal. One adrenal from each animal was placed into an electrolyte replacement solution (Sterofundin®) on ice for in vitro analysis. Urine samples were stored at $-30^\circ$C and analysed for sodium and potassium excretion on the last 2 days.

**In vitro experiments**

Cell suspensions from one freshly isolated adrenal gland from each animal were prepared by the collagenase/deoxyribonuclease dispersion method described by Eckhoff et al. (17), including some modifications. Adrenal glands were cut into small pieces and transferred into a Krebs–Ringer bicarbonate buffer containing bovine serum albumin and glucose (KRBGA) at 37°C. The incubate was stirred, gassed with a mixture of 95% air and 5% CO$_2$ and treated for 45 min with 1.5 mg/ml collagenase and for 15 min with additional 0.4 mg/ml deoxyribonuclease. After repeated washing with KRBGA, the adrenal cells were counted and cell viability was determined by the trypan blue exclusion test (always >90%).

Samples of cell suspension (1 ml) were distributed in wells of a cell culture plate that was placed in a flat metal box shaken in a 37°C water bath and gassed with 95% air and 5% CO$_2$. After incubation, the cell suspension was centrifuged at 3600 g and 4°C for 10 min and the supernatant was kept frozen at $-20^\circ$C until analysis.

To test endogenous aldosterone and cortisol production, $1 \times 10^5$ adrenal cells/ml KRBGA were incubated in triplicate or quadruplicate without stimuli or with ACTH (10$^{-10}$ mol/l) or 9.6 mmol/l K$^+$ for 5 h. The prolonged incubation period was chosen in order to be able to measure possible reductions of steroid formation in vitamin C-deficient animals. Aldosterone and cortisol concentrations in the incubation medium were measured by direct radioimmunoassay. To investigate [3H]deoxycorticosterone (DOC) metabolism, $5 \times 10^5$ adrenal cells/ml were dispersed in KRBGA containing 20 µg/ml gentamicin and 20 IU/ml nystatin. After a precollection period of 2 h, an ethanolic solution of 42 KBq [3H]DOC was added to the cell suspension. The final [3H]DOC concentration in the incubation medium was 17 nmol/l and the final ethanol concentration was 1%. The incubation was carried out for 24 h. The supernatants obtained by centrifugation were used for solid-phase extraction and for HPLC with on-line measurement of tritium-labelled steroid metabolites.

**Radioimmunoassays**

The plasma steroids cortisol and aldosterone were determined in solid-phase radioimmunoassays (coated tubes) by the use of commercial kits (Coat-A-Count) distributed by Hermann Biermann Diagnostica (Bad Nauheim, Germany). The intra-assay variabilities were 4.2% for cortisol and 3.4% for aldosterone, and the interassay variabilities were 6.9% and 9.1%, respectively. For cortisol the sensitivity was 27.6 nmol/l, and for aldosterone it was 0.069 nmol/l. Plasma ACTH was analysed by an immunoradiometric assay (IRMA) from Nichols Institute (San Capistrano, CA). Guinea pig ACTH showed 100% cross-reactivity with human ACTH in this assay. Only intact ACTH$_{1-39}$ is measured by this double antibody assay, with a sensitivity of 1.21 pmol/l, an intra-assay variability of 3% and an interassay variability of 9.8%. Plasma renin activity (PRA) was determined by the measurement of angiotensin I formed after incubation of plasma samples at 37°C using a commercial radioimmunoassay kit (Du Pont Company, Billerica, MA). The intra-assay variability (pool with PRA of 9.7 ng/ml/h) is declared to be 5.2% and the sensitivity approximately 30 pg/ml.

Cortisol and aldosterone in supernatants of cell incubates were determined using a modified method of Schönshöfer (18), omitting chromatography prior to the radioimmunoassay and using commercial anti-cortisol-3-(CMO)HSA or anti-aldosterone-3-(CMO)BSA antibodies. Cross-reactivities with the aldosterone antiserum were all declared to be <0.4%. Free and antibody-bound [3H]-labelled steroids were separated using dextran-coated charcoal. Intra-assay variabilities were 4.32% for aldosterone-RIA and 4.42% for cortisol-RIA; interassay variabilities were 9.05% and 15.7%, respectively. The least detectable concentrations were 46 pg/ml for aldosterone and 76 pg/ml for cortisol.

**Measurement of vitamins C and E**

Vitamin C was analysed in guinea pig serum, liver and the adrenal gland by the photometric method of Zannoni et al. (19). The procedure was modified by using an aqueous solution containing both 1% 2,2'-bipyridine and 10.63% orthophosphoric acid. The interassay variability of a human serum pool was 7.9% and the intra-assay variability was 4.8%. The minimum detection level for serum ascorbic acid was 10.25 µmol/l, for adrenal tissue it was 25 nmol/g and for liver tissue it was 15 nmol/g. Vitamin E was determined as α-tocopherol by reversed-phase HPLC and UV detection at 290 nm in serum, liver and the adrenal gland, using a C$_{18}$ column (Spherisorb S5 ODS II 5 µm, 250 × 4 mm and a 5 × 4 mm precolumn) and 2 ml/h methanol/water (96:4%) (20).

The extraction procedure of serum and tissue tocopherol was performed according to the slightly modified method of Zaspel and Csallany (16). Serum (300 µl) was mixed with 12.5 µg of α-α-tocopheryl acetate (50 µl), 3 ml of acetone and 0.45 g of anhydrous sodium sulphate. The dried residue was redissolved in 300 µl of methanol and 100 µl was injected onto the HPLC column.

Adrenal tissue (50–100 mg) was homogenized in 2 ml of acetone, centrifuged and re-extracted twice. Liver tissue (100 mg) was homogenized with 2 ml of
acetone and re-extracted once. The supernatants were fully dried under nitrogen in the darkness. After dissolving the residues of adrenal extracts in 300 µl of acetone or the liver extracts in 300 µl of methanol, the samples were centrifuged, the supernatants mixed with the same volume of the mobile phase and 100-µl samples were injected. The standards d-α-tocopherol and d-α-tocopheryl acetate were purified by HPLC before use.

The interassay variability of a human serum pool was 4.7% and the intra-assay variability was 5.9%.

**Analysis of in vitro metabolites of [3H]DOC**

Incubates were deproteinized and subjected to solid-phase extraction with Sep-Pak® C18 cartridges as described by Eckhoff et al. (17). The eluates were evaporated to dryness under air, redissolved in the mobile phase and mixed with a solution of ten steroids. Samples were separated by normal-phase HPLC using a diol column at 40°C and running a hexane/2-propanol gradient at 1.3 ml/min. A linear gradient with the following segments was run: start, 6.25% 2-propanol; at 15 min. 10%; 30 min. 10%; 35 min 21.25%; 42 min. 21.25%; 43 min. 25%; 44 min. 25% 2-propanol. Tritiated metabolites were quantified on line by an HPLC radioactivity monitor. They were identified by comparing the retention times of the radiochromatogram with those of the UV absorbance of non-radioactive steroids in the added mixture. Aldosterone appeared in two different peaks, presumably representing two tautomeric forms. Areas under the curve from these two peaks were summed up.

Sodium and potassium in serum and urine were measured by flame photometry (Instrumentation Laboratory 973, Milan, Italy).

**Statistics**

Data were expressed as means ±SEM. For statistical analysis, the Mann–Whitney–Wilcoxon U test was used.

**Results**

**Degree of vitamin C depletion**

In a pilot study we confirmed results published by Odumosu et al. (1) that vitamin C depletion lowers ascorbic acid concentrations in parallel in the adrenal and the liver (19). Vitamin C depletion for 15 days decreased adrenal vitamin C concentration to less than 1/20 from 3.5–7.2 to 0.2–0.3 µmol/g wet weight. Because we conserved the adrenals in the main experiment for in vitro studies of aldosterone synthesis, we controlled vitamin C depletion by measuring the concentration of ascorbic acid in serum and liver. After 15 days of vitamin C depletion, serum ascorbic acid had decreased to about 1/3 and liver vitamin C to about 1/10 compared to animals receiving a normal vitamin C diet (Table 1). At this time, very few animals showed visible symptoms of scurvy, such as weight loss and salivation, whilst almost all animals had typical

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<th>Table 1. Vitamin C and E levels, plasma ACTH, plasma renin activity (PRA) and serum electrolytes on the day of sacrifice of animals of groups A–F.</th>
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*Means ±SEM from 7–9 animals per group are given; *p < 0.05. **p < 0.01 and ***p < 0.005 compared with respective controls: \*p < 0.01 compared with group E: n.d. = not done. Urinary sodium and potassium levels represent the means of two 24-h collection periods (days 14 and 15 on the diet).
haemorrhages, especially around the stifle joints, at post-mortem examination. No significant changes in vitamin E concentration in serum or liver occurred upon vitamin C depletion (Table 1).

**Steroid secretion and [3H]DOC metabolism**

As shown in Fig. 1, plasma cortisol increased slightly (p < 0.05) in the vitamin C-depleted group B compared with group A. Cortisol secretion in vitro (Fig. 2), however, was slightly diminished in group B. Because plasma ACTH was not different from the control in the experimental groups (Table 1), the rise in plasma cortisol in group B is probably not due to cortisol hypersecretion.

Three days of ACTH treatment led to an almost six-fold increase of plasma cortisol (Fig. 1). In vitro cortisol secretion also increased markedly (Fig. 2). The ACTH-induced increase in cortisol secretion was hardly affected by vitamin C depletion (group D).

Sodium depletion in group E led to a threefold increase in plasma aldosterone concentration compared with group A (p < 0.001), while in vitro aldosterone

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**Fig. 1.** Plasma cortisol (a) and aldosterone (b) in six groups of guinea pigs: A = controls, B = vitamin C-depleted, C = ACTH-treated, D = ACTH-treated and vitamin C-depleted, E = sodium-depleted, F = sodium-depleted and vitamin C-depleted. Animals received special diets for 15 days. Adrenocorticotrophin injections (groups C and D) were given during the last 3 days before sacrifice. Values are means ±SEM for 7–9 animals per group; *p < 0.05, **p < 0.01 and ***p < 0.001.

**Fig. 2.** In vitro cortisol (a) and aldosterone (b) secretion of adrenal cell suspensions from six groups of guinea pigs treated as described in Fig. 1. Isolated adrenal cells (10^5/ml) were incubated in triplicate or quadruplicate for 5 h without in vitro stimuli. Steroids in the incubation medium were determined by direct RIAs. Values are means ±SEM for 7–9 animals per group; *p < 0.05, **p < 0.01 and ***p < 0.001.
secretion increased to about 170% of control. The significant increase in plasma aldosterone in group E was accompanied by a modest rise of PRA only (Table 1). In the vitamin C-depleted group B, plasma aldosterone was slightly higher than the control but the increment of plasma aldosterone and of in vitro aldosterone secretion in response to sodium depletion was abolished completely by simultaneous vitamin C depletion (group F versus B). This unresponsiveness of aldosterone to sodium deficiency in vitamin C-depleted animals is not due to differences in plasma ACTH, PRA or serum K⁺ between groups E (sodium depletion) and F (additional vitamin C depletion), as shown in Table 1. Although groups E and F received the same concentration of sodium in their diets, sodium excretion on days 14 and 15 was almost twice as high in group F than in group E (p < 0.01, Table 1). Animals of group F were obviously less efficient in renal sodium conservation than those of group E. This is also reflected by weight differences in the groups. While animals in group F gain 17 ± 8 g of weight during the 15 days on the diet, those of group E had no gain in weight (∆3 g).

Sodium deficiency did not lead to a significant increase in the percentage conversion of [³H]DOC to [³H]18-OH-corticosterone or [³H]aldosterone (Fig. 3). However, vitamin C deficiency in sodium-depleted animals led to a small but significant reduction in the percentage conversion of [³H]DOC to [³H]aldosterone.

As mentioned before, ACTH treatment greatly enhanced cortisol secretion. In contrast, plasma aldosterone, in vitro secretion of aldosterone and the conversion of [³H]DOC to [³H]18-OH-corticosterone and [³H]aldosterone were depressed significantly by 3 days of ACTH pretreatment.

Corticosterone secretion of isolated adrenal cells was stimulated by 10⁻¹⁰ mol/l ACTH (added in vitro) to an average of 900% of control without significant differences between the groups. Aldosterone secretion in vitro was stimulated by ACTH or by 9.6 mol/l potassium to an average of 170% and 190%, respectively, without significant differences in percentage stimulation between groups A–F (data not shown).

Discussion

Vitamin C depletion was monitored in serum and liver in all experiments, and also in the adrenal gland in pilot studies. Ascorbate concentrations in tissues from guinea pigs with a normal vitamin C diet and from vitamin C-depleted animals are similar to those reported by Chakraborty et al. (21). Vitamin C depletion had no influence on serum or tissue concentrations of vitamin E. This finding is in contrast to in vitro studies, in which vitamin C was shown to protect or regenerate vitamin E (12). Our results are in keeping with other recently published in vivo experiments in guinea pigs on different vitamin C diets (21–23). Although changes in vitamin E concentration in relevant cellular compartments (i.e. inner mitochondrial membranes in adrenocortical cells) cannot be excluded, our results do not support the idea of an indirect protection against oxygen radical species by vitamin C via the regeneration of vitamin E.

![Fig. 3. In vitro conversion of [³H]deoxycorticosterone into [³H]-corticosterone (a), [³H]18-hydroxycorticosterone (b) and [³H]aldosterone (c) by adrenal cell suspensions from guinea pigs. Animal groups were treated as described in Fig. 1. After a 24-h incubation period of 5 x 10⁴ adrenal cells per ml. metabolites were extracted, separated by HPLC and measured by a radioactivity monitor. Data are expressed as percentage recovered activity; 100% is the total activity of one radiochromatogram. Values are means ± SEM for 7–9 animals per group; * p ≤ 0.05, ** p ≤ 0.01 and *** p ≤ 0.001.](image-url)
Plasma concentrations of cortisol and aldosterone in our control animals are high compared to other species. This species difference has been reported before (24–26) and may be explained in part by a low affinity of the glucocorticoid receptor to cortisol. The affinity of the guinea pig mineralocorticoid receptor (type 1) to aldosterone is the same as in the rat and may not explain high aldosterone plasma concentrations in this species (27). However, PRA was found to be about 3–4 times higher in the control guinea pigs than in man (Table 1).

Stimulation of aldosterone secretion by sodium depletion, as indicated by increased aldosterone plasma levels and by aldosterone secretion of isolated adrenal cells, is abolished by vitamin C depletion. To our knowledge, this is the first report concerning a role of the vitamin C status in vivo on aldosterone synthesis. Because prolonged vitamin C depletion leading to scurvy was found to be associated with a rise in plasma ACTH (16), which could influence aldosterone secretion, we attempted to study aldosterone secretion in a state of vitamin C depletion before scurvy developed. Consequently, plasma ACTH levels of animals on vitamin C-deficient and control diets were not significantly different. Therefore, changes in endogenous ACTH are obviously not the mechanism by which the effect of sodium deficiency on aldosterone is blunted in the state of vitamin C depletion.

To our surprise, PRA increased modestly only in the sodium-depleted group E compared with group A consuming a “normal sodium diet”. In the latter group, PRA was markedly higher than in the human on a free sodium diet (0.5–4.0 ng·ml⁻¹·h⁻¹) as measured with the same assay. Thus, the marked rise of plasma aldosterone in group E compared with group A cannot be explained fully by an activation of the renin–angiotensin system, nor were changes in serum potassium encountered that could have stimulated aldosterone secretion in group E. It is, therefore, likely that sensitization of the zona glomerulosa towards angiotensin II by sodium deficiency per se (28, 29) was involved in aldosterone stimulation in group E.

Because PRA and serum potassium were not different between group E and group F (sodium plus vitamin C deficiency), vitamin C deficiency blunted the response of aldosterone secretion to sodium deficiency probably by an intra-adrenal mechanism, and not by effects on humoral aldosterone-modulating factors.

The conversion of tritiated DOC to aldosterone was not increased by sodium depletion. Oort and Müller showed a modest but significant increase in the formation of aldosterone from DOC in rat capsular adrenal tissue incubations (30). The different species, a different degree of sodium depletion, the higher substrate concentration (50 µmol/l) in the experiments of Oort and Müller (30) and the high percentage of zona glomerulosa cells in their study may explain the different results.

The conversion of tritiated DOC to aldosterone but not to corticosterone was reduced significantly in group F compared with group E. This can be interpreted in the sense that vitamin C deficiency affected the last step of aldosterone synthesis, which is catalysed in man and in the rat by aldosterone synthase (11, 12), while it is still unknown whether this enzyme exists in the guinea pig or whether this species synthesizes aldosterone with the aid of the 11-hydroxylase like the cow (13). Chakraborty et al. (21) recently reported on a great increase of lipid peroxidation in the adrenals from vitamin C-depleted guinea pigs. Whether the effect of vitamin C deficiency on the late steps of aldosterone synthesis is due to enhanced lipid peroxidation, to a role of vitamin C in mitochondrial electron transport (31) or to other mechanisms has to be elucidated in later studies.

The marked suppression of aldosterone secretion in vivo and in vitro by 3 days of ACTH injections is similar to the effect of prolonged intravenous ACTH infusion in man (32). Provencher et al. observed a stimulation or slight suppression of plasma aldosterone by 10 days of ACTH injection in guinea pigs (24). Different dosage and timing and the use of non-depot ACTH may explain their different results. The more constant plasma ACTH levels resulting from two daily injections of depot ACTH in our study may be closer to the situation of continuous prolonged ACTH infusion in man, resulting in a suppression of aldosterone secretion. The same suppressive effect of prolonged ACTH administration has been observed in other species (33–35). It has been shown in the rat that aldosterone synthase gene expression is inhibited by prolonged in vivo ACTH treatment (36, 37). Our finding that in ACTH-treated groups C and D the conversion of tritiated DOC to 18-OH-corticosterone and aldosterone but not to tritiated corticosterone was markedly inhibited by in vivo ACTH treatment, is in favour of an inhibitory mechanism affecting the last step of aldosterone synthesis also in the guinea pig. Vitamin C depletion in no way modulated the effect of in vivo ACTH treatment on aldosterone secretion. Because our data lead to the conclusion that both vitamin C depletion and ACTH treatment inhibit the last step of aldosterone synthesis, such an effect could not be expected.

A further observation of interest is the significant rise of plasma cortisol in group B (vitamin C depletion), while in vitro cortisol synthesis was reduced, albeit not significantly. This finding is easiest explained by decreased hepatic cortisol metabolism. As far as we are aware, the effect of vitamin C deficiency on cortisol metabolism or the activity of cortisol-metabolizing liver enzymes has not been studied so far.

In conclusion, we have shown for the first time that vitamin C has a permissive role in the increase of aldosterone secretion in response to sodium deficiency in the guinea pig. The effect of vitamin C does not seem to be mediated by humoral aldosterone-modulating factors. An involvement of the last enzymatic step of
aldosterone synthesis is likely. Further experiments are necessary to clarify the mechanism(s) of action.

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