

EFFECT OF VITAMIN E-DEFICIENCY ON RENIN RELEASE FROM RENIN GRANULES

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This study was undertaken to investigate the effects of vitamin E-deficiency on renin release from isolated renin granules. Male Wistar rats were fed either a control or a vitamin E-deficient diet. Renin granules were prepared from the kidney cortex homogenate by a discontinuous sucrose density gradient centrifugation. Renin activity was measured by radioimmunoassay and lipid peroxidation was estimated by means of the thiobarbituric acid test. The intake of vitamin E-deficient diet for 4 weeks resulted in a decrease in α -tocopherol content in renin granules, accompanied by an increased level of endogenous lipid peroxides. When the renin granules were incubated at 37°C, the rate of renin release from the granules in vitamin E-deficient group was significantly higher than that in the control group. These results indicate that vitamin E exists in renin granule membranes and functions in maintenance of membrane integrity by blocking the lipid peroxidation.

Keywords —rat renin granules; renin release; vitamin E content; vitamin E-deficiency; lipid peroxidation

INTRODUCTION

It is well known that the membranes of subcellular organelles contain a large amount of unsaturated lipids. The peroxidation of unsaturated lipids is considered to induce membrane damage and impairment of cellular function. On the other hand, numerous studies have shown that vitamin E serves an important antioxidant role in subcellular membranes by blocking the lipid peroxidation. Recently, we reported that renin release from renin granules was markedly stimulated by the addition of ascorbic acid or ferrous ions, accompanied by increased formation of lipid peroxides in renin granules.¹⁾ The present study was undertaken to further investigate the relation between renin release and lipid peroxidation under vitamin E-deficient conditions.

MATERIALS AND METHODS

Male Wistar rats weighing 70–80 g were used. For 1 week prior to the experiments, the rats were fed a standard diet and received tap

water *ad libitum*. The animals were separated into control and experimental groups. The experimental animals were maintained on a vitamin E-deficient basal diet, while the control animals were given a control diet prepared by the addition of 2 mg of *dl*- α -tocopheryl acetate to 100 g of the basal diet for 4 weeks. All rats were sacrificed, and the vitamin E status was assessed by the erythrocyte hemolysis test with dialuric acid.²⁾ The kidney cortex was removed from the medulla and homogenized with 0.45 M sucrose. Renin granules were prepared from the homogenate by a discontinuous sucrose density gradient centrifugation according to the method described previously.³⁾ First, renin granules were tested for α -tocopherol content and lipid peroxide level. α -Tocopherol content was determined by fluorometric method of Taylor *et al.*⁴⁾ Lipid peroxides were measured in terms of the formation of 2-thiobarbituric acid-reacting substances, presumed to be malondialdehyde (MDA), by means of a fluorometric assay described by

Ohkawa *et al.*⁵⁾ Protein content was determined by the method of Lowry *et al.*⁶⁾ Next, the effects of vitamin E depletion on renin release from the granules were investigated. Half ml of the renin granule fraction (0.45–0.50 mg protein) was suspended in 0.5 ml of 0.1 M Tris-HCl buffer (pH 7.0) containing 0.15 M KCl, and incubated at 37°C for 0–120 min. The incubation medium was separated into the supernatant and sediment by centrifugation at $105000 \times g$ for 60 min. Renin activity in the sample was measured by radioimmunoassay of generated angiotensin I after incubation with partially purified rat renin substrate, which was prepared by our method previously reported.⁷⁾ Statistical significance was determined by means of Student's *t*-test.

RESULTS AND DISCUSSION

Throughout the experimental period, no significant difference could be detected in average body weight or kidney weight per 100 g of body weight between the control and experimental

rats. The animals maintained on the control and vitamin E-deficient diets for 4 weeks showed 0–0.5% and 90–95% hemolysis against dialuric acid, respectively. Further, vitamin E-deficiency was examined by assaying for plasma α -tocopherol content. Mean α -tocopherol content in plasma of vitamin E-deficient rats was 0.15 mg/dl, being approximately 12% of the control value.

It has been shown that α -tocopherol is located in the membranes of subcellular organelles in various tissues.⁴⁾ In the present study, renin granules of control rats contained $0.11 \pm 0.02 \mu\text{g}$ α -tocopherol per mg protein (Fig. 1). In one other experiment, we determined the contents of α -tocopherol in mitochondria and microsomes prepared from the kidney cortex homogenate of control rats. Mitochondria and micro-

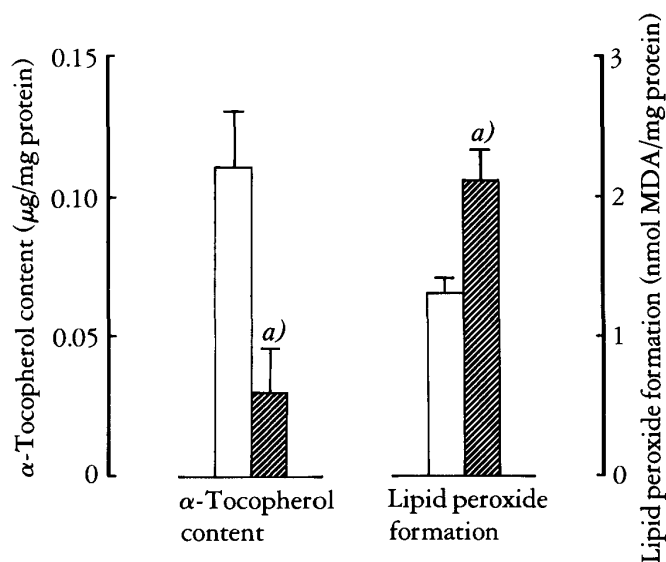


FIG. 1. α -Tocopherol Content and Lipid Peroxide Formation in Renin Granules from Control and Vitamin E-Deficient Rats

Each column represents the mean of four separate experiments, and vertical bars indicate S.E. of the mean. Values are significantly different from each control value (a) $p < 0.05$.

□ control, ▨ vitamin E-deficient.

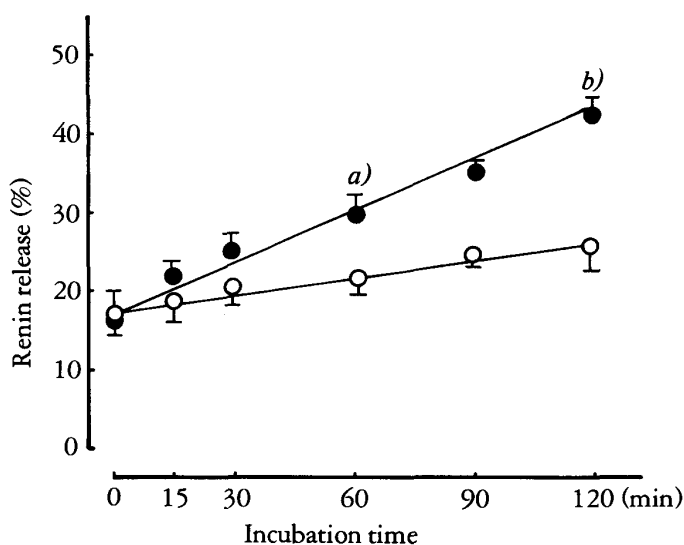


FIG. 2. Time Course of Renin Release during Incubation of Renin Granules from Control and Vitamin E-Deficient Rats

Total renin was taken to be sum of renin content in the supernatant and sediment, and the amount of renin release during incubation was expressed as percentage of total renin. Each point represents the mean of four separate experiments, and vertical bars indicate S.E. of the mean. Values are significantly different from each control value a) $p < 0.05$, b) $p < 0.01$.

○ — ○ control, ● — ● vitamin E-deficient.

somes contained 0.10–0.15 μg α -tocopherol per mg protein, indicating that α -tocopherol contents in these subcellular organelles were the same as those in renin granules. On the other hand, the intake of vitamin E-deficient diet for 4 weeks resulted in a marked decrease in α -tocopherol content of renin granules, being 0.03 ± 0.01 μg per mg protein. Furthermore, we examined the level of endogenous lipid peroxides in renin granules. Vitamin E-deficiency caused about 60% increase in lipid peroxide level in renin granules compared with the control value, which was 1.30 ± 0.10 nmol MDA per mg protein (Fig. 1). These findings indicate that vitamin E acts as an antioxidant in the membranes of renin granules.

Several investigations have demonstrated the close relationship between lipid peroxidation and increased permeability in subcellular organelles such as mitochondria⁸⁾ and lysosomes.⁹⁾ In the present study, we determined whether renin release from the granules was affected by vitamin E-deficiency. Fig. 2 shows the time course of renin release during incubation of renin granules at 37°C. The rate of renin release in vitamin E-deficient group was significantly higher than that in the control group, although the time-dependent increases were observed in both groups.

From the above results, it is assumed that vitamin E involved in renin granules functions in maintenance of membrane integrity by inhibiting the lipid peroxidation. Further studies on the effects of *in vivo* repletion of vitamin E are being conducted in our laboratory, and the

details will be reported in the near future.

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