STIMULATION OF RENIN RELEASE FROM RAT KIDNEY COR-TICAL SLICES BY VITAMIN E-DEFICIENCY

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(Received August 10, 1983)

Effect of vitamin E-deficiency on renin release was examined with rat kidney cortical slices. Male Wistar rats were fed either a control or a vitamin E-deficient diet for 4 weeks. When kidney cortical slices were incubated in a Krebs-Ringer's bicarbonate solution (pH 7.4) at 37°C, the rate of renin release into the incubation medium in vitamin E-deficient group was significantly higher than that in the control group. However, dietary supplementation of α-tocopheryl acetate (TOCA) or N,N'-diphenyl-p-phenylenediamine (DPPD) to the vitamin E-deficient rats for 5 d suppressed the stimulation of renin release from kidney cortical slices by vitamin E-deficiency. On the other hand, the release of protein, acid phosphatase and alkaline phosphatase during incubation of kidney cortical slices was not affected by vitamin E-deficiency or supplementations of TOCA and DPPD. These findings indicate that vitamin E-deficiency specifically stimulates renin release from kidney cortical slices and this effect is attenuated by the dietary supplementation of TOCA or DPPD.

Keywords — rat kidney cortical slices; renin release; vitamin E-deficiency; dietary supplementation of α-tocopheryl acetate; dietary supplementation of N,N'-diphenyl-p-phenylenediamine

INTRODUCTION

In recent years, lipid peroxidation has been considered to be an important factor which causes the impairment of biomembrane integrity. On the other hand, vitamin E serves an important antioxidant role in cellular and subcellular membranes by blocking the peroxidation of polyunsaturated fatty acid constituents.1–3) In the previous studies, we found that renin release from renin granules was markedly enhanced by ferrous ions or ascorbic acid, accompanied by increased formation of lipid peroxides in the renin granule fraction.4) Furthermore, we demonstrated that the rate of renin release from the granules was stimulated by vitamin E-deficiency.5) The present study was carried out to further investigate the effect of dietary vitamin E-deficiency on renin release from kidney cortical slices.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats (70–80 g). For at least 1 week before the study, the rats were fed a standard laboratory rat chow and received tap water ad libitum. The animals were divided into the 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 (TOCA) to 100 g of the basal diet for 4 weeks. Subsequently, parts of vitamin E-deficient animals received dietary supplementation of TOCA or N,N'-diphenyl-p-phenylenediamine (DPPD) for 5 d. The supplemental diet of TOCA or DPPD was prepared by the addition of 40 mg of TOCA or 80 mg of DPPD to 100 g of vitamin E-deficient basal diet. Vitamin E status of each animal was checked by the hemolytic action of dialuric acid on erythrocytes.6) Under pentobarbital anesthesia (30 mg/kg, i.p.), both kidneys were removed, decapsulated, and immediately placed in a cold physiological saline. The cortex was sectioned into
thin slices with a razor blade under low temperature and rinsed thoroughly with cold physiological saline. Krebs-Ringer’s bicarbonate solution (pH 7.4) was used as an incubation medium which had the following compositions in mM: NaCl, 119; KCl, 4.7; CaCl₂, 2.5; KH₂PO₄, 1.2; MgSO₄, 1.2; NaHCO₃, 25; and glucose, 11.1. Three slices (70–100 mg) were placed in each flask that contained 3 ml of incubation medium, which had been equilibrated previously at 37°C with a mixture of O₂ (95%) and CO₂ (5%). The flasks were placed in a metabolic incubator maintained at 37°C and flushed continuously with the gas mixture. After preincubation of slices for stabilization for 15 min, the medium was discarded. Subsequently, the slices were incubated for five consecutive 15 min periods in 3 ml of fresh incubation medium. At the end of each period the medium was aspirated and centrifuged at 3000 rpm for 10 min. The supernatant and kidney cortical tissues were analyzed for the protein content and activities of renin, acid phosphatase and alkaline phosphatase. Protein content was determined by the method of Lowry et al. Renin activity was measured by radioimmunoassay of angiotensin I produced after incubation with rat renin substrate which was prepared by our method previously reported. The activities of acid phosphatase and alkaline phosphatase were assayed according to the method of Wright et al. All results were expressed as means ± S.E. Statistical significance was determined by means of Student’s t-test.

RESULTS AND DISCUSSION

The animals maintained on a vitamin E-deficient diet for 4 weeks showed 94.0±3.9% erythrocyte hemolysis. The hemolysis of these animals was restored to the control value (0.5±0.2%) by dietary supplementation of TOCA or DPPD for succeeding 5 d.

Since the renin content of kidney cortical slices from different rats showed a wide variation, we first determined the relationship between kidney cortical renin content and basal renin release rate in 25 experiments using kidney cortical slices of different normal rats. Linear regression analysis yielded an r value

![Graph showing Time Course of Renin Release during Incubation of Kidney Cortical Slices](image)

**FIG. 1. Time Course of Renin Release during Incubation of Kidney Cortical Slices**

The rate of renin release during incubation was expressed as percentage of kidney cortical renin content. Each point represents the mean of eight separate experiments, and vertical bars indicate S.E. of the mean. a) Values are significantly different from each control value (p < 0.001). b) Values are significantly different from each value of vitamin E-deficient group (p < 0.05). ○ — ○, control; ● — ●, vitamin E-deficient; △ — △, TOCA supplemented; ▲ — ▲, DPPD supplemented. The inset shows the kidney cortical renin content (ng of angiotensin I per hour per mg of wet tissue) of four groups. Each column in the inset represents the mean ± S.E. of eight separate experiments. [ ] : control; [ ] : vitamin E-deficient; [ ] : TOCA supplemented; [ ] : DPPD supplemented.
equal to 0.800 (p < 0.001), which indicated that the basal renin release rate depended directly on the amount of renin stored in the kidney cortex. Accordingly, the rate of renin release during incubation was expressed as a percentage of renin content of kidney cortical slices.

When kidney cortical slices were incubated at 37°C, renin release was stable during the 75-min incubation time, as shown in Fig. 1. In the control group, renin was released at the average rate of approximately 3% of kidney cortical content per 15 min, and the accumulated amount of renin released during incubation for 75 min was 14.7 ± 0.57% of kidney cortical content. In the vitamin E-deficient group, renin release was significantly higher compared with the control group and the accumulated amount of renin released during incubation for 75 min was 21.01 ± 1.83% of kidney cortical content. Recently, we found that the increases in renin release and lipid peroxidation in the renin granule fraction due to vitamin E-deficiency were restored to the control value by dietary supplementation of TOCA or DPPD, which has an antioxidative ability. In the present study, the effects of dietary supplementation of these agents on renin release were examined with kidney cortical slices of vitamin E-deficient rats. The high rate of renin release from the slices due to vitamin E-deficiency was significantly suppressed by the supplementation of TOCA or DPPD. However, no significant difference could be detected in the average renin content of kidney cortical tissues among the control and three experimental groups, as shown in the inset of Fig. 1.

Previously, De Vito et al. suggested that the release of renin from rat kidney slices was an active process, on the basis of findings that the percentage of renin release per hour was higher than the rates of release of acid phosphatase and protein. Similarly, we found that the percentage of renin release was much higher than the rates of release of acid phosphatase, alkaline phosphatase and protein during incubation of kidney cortical slices of control rats for 75 min (Table I). Although the rate of renin release was augmented by vitamin E-deficiency and this effect was attenuated by the supplementation of TOCA or DPPD, no significant difference was observed in release rates of acid phosphatase, alkaline phosphatase and protein among the control and three experimental groups.

These results indicate that vitamin E-deficiency for 4 weeks specifically stimulates renin release from kidney cortical slices, probably due to increased release of renin from renin granules in the juxtaglomerular cells and that the dietary supplementation of TOCA or DPPD would suppress the increased release of renin from kidney cortical slices by inhibiting lipid peroxidation of phospholipids in renin granules.

**Acknowledgement**  We wish to thank the

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<tr>
<th>Experimental group</th>
<th>Renin</th>
<th>Acid phosphatase</th>
<th>Alkaline phosphatase</th>
<th>Protein</th>
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<tr>
<td>Control</td>
<td>14.7±0.57</td>
<td>8.72±0.25</td>
<td>6.80±0.44</td>
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<td>Vitamin E-deficient</td>
<td>21.7±1.03</td>
<td>8.49±0.42</td>
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<td>8.90±0.80</td>
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<td>TOCA supplemented</td>
<td>17.1±0.51</td>
<td>8.92±0.42</td>
<td>6.78±0.43</td>
<td>8.82±0.41</td>
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<tr>
<td>DPPD supplemented</td>
<td>17.4±1.09</td>
<td>8.06±0.26</td>
<td>6.63±0.53</td>
<td>8.30±0.53</td>
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The amounts of enzymes and protein released during incubation for 75 min were expressed as a percentage of the content of kidney cortical slices. Values represent means ± S.E. of eight experiments. a) Value is significantly different from the control value (p < 0.001). b, c) Values are significantly different from the vitamin E-deficient group (b, p < 0.01; c, p < 0.05).
Eisai Co., Ltd., Tokyo, Japan for providing the vitamin E-deficient diet and dl-α-tocopheryl acetate.

REFERENCES


