[Chem. Pharm. Bull. 32(5)2033—2035(1984)]

Effects of Ascorbic Acid and Ferrous Ions on Renin Release from Renin Granules of Vitamin E-Deficient Rats

SHIRO MORIMOTO,* YUKIHIRO OHNO, YASUO MATSUMURA, NOBUAKI MIYAWAKI and YASUHISA MIZUNOYA

Osaka College of Pharmacy, 2–10–65 Kawai, Matsubara, Osaka 580, Japan

(Received August 31, 1983)

This study was undertaken to investigate the effects of ascorbic acid (ASA) and ferrous ions on renin release from renin granules of vitamin E-deficient rats. Male Wistar rats were maintained on a vitamin E-deficient diet for 4 weeks. The renin granule fraction was prepared from the kidney cortex homogenate by discontinuous sucrose density gradient centrifugation. When the renin granule fraction was incubated at 37 °C for 30 min in the presence of ASA or ferrous ions, renin release from the granules in vitamin E-deficient rats was significantly higher than that in the case of control rats. Further, lipid peroxide formation was greatly enhanced in the renin granule fraction. However, dietary supplementation of *dl*-α-tocopheryl acetate (40 mg/100 g diet) or *N,N'*-diphenyl-*p*-phenylenediamine (80 mg/100 g diet) to the vitamin E-deficient rats for 5 d suppressed the simultaneous increases in renin release and lipid peroxide formation induced by ASA or ferrous ions. These results indicate that renin granules of vitamin E-deficient rats are more susceptible to renin relase by ASA and ferrous ions than are those of control rats, and that vitamin E functions to maintain the membrane integrity of renin granules by inhibiting lipid peroxidation.

Keywords——rat renin granule; vitamin E deficiency; renin release; lipid peroxide formation; ascorbic acid; ferrous ion; *dl*-α-tocopheryl acetate; *N,N'*-diphenyl-*p*-phenylenediamine

In has been reported that the lysis of cells or subcellular structures is frequently attributable to peroxidation of the lipid components of their membranes.[1] On the other hand, vitamin E has an important role as an antioxidant in cellular and subcellular membranes by blocking the peroxidation of polyunsaturated fatty acid constituents of essential lipids.[2,3] In the previous study, we demonstrated that renin release from renin granules was markedly enhanced by ascorbic acid (ASA) or ferrous ions, accompanied by increased formation of lipid peroxides in the renin granule fraction.[4] Furthermore, we reported that the rate of renin release from the granules was increased by vitamin E deficiency.[5] The present study was designed to further investigate the effects of ASA and ferrous ions on renin release from the granules of vitamin E-deficient rats.

Materials and Methods

Animal Experiments——Male Wistar rats weighing 70—80 g were used. For 1 week before the study, the rats were fed a standard laboratory rat chow and provided with tap water *ad libitum*. The animals were divided into control and experimental groups. The experimental animals were given a vitamin E-deficient diet, while the control animals were maintained on 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, some of the vitamin E-deficient animals received dietary supplementation of TOCA or *N,N'*-diphenyl-*p*-phenylenediamine (DPPD) for 5 d. The vitamin E-deficient basal diet and supplemental diets of TOCA (40 mg/100 g diet) and DPPD (80 mg/100 g diet) contained the components described in our previous paper.[6]

Preparation and Incubation of the Renin Granule Fraction——Under pentobarbital anesthesia (40 mg/kg), both kidneys were removed and immediately placed in a cold physiological saline. The cortex was sectioned into thin slices

and homogenized with cold 0.45 M sucrose. The renin granule fraction was prepared from the homogenate according to our method described previously.[7] One-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. ASA and ferrous sulfate were dissolved in the same buffer and added to the renin granule fraction prior to the incubation. The suspension was incubated at 37 °C for 30 min, and then centrifuged at 105000 *g* for 60 min. The sediment was resuspended in Tris buffer and analyzed for renin activity, lipid peroxides and protein content. The supernatant was analyzed for renin activity.

Renin Assay and Measurements of Lipid Peroxides and Protein Contents——Renin activity was measured by radioimmunoassay of angiotensin I (AI) produced after incubation with rat renin substrate,[8] and expressed as μg of AI/ml/h. Total renin was taken to be the sum of renin contents in the sediment and supernatant, and the amount of renin release during incubation was expressed as a percentage of total renin. Lipid peroxides were measured in terms of the formation of 2-thiobarbituric acid-reacting substances, presumed to be malondialdehyde (MDA), by means of the fluorometric assay described by Ohkawa *et al.*[9] The protein contents were determined by the method of Lowry *et al.*[10] with modifications as described by Bensadoun and Weinstein[11] to eliminate the interference by Tris buffer. All results were expressed as means ± S.E. Statistical significance was determined by means of Student's *t*-test.

Results and Discussion

No significant difference could be detected in total renin activity (16.54 ± 1.21 μg AI/h) or total protein content (0.96 ± 0.04 mg) per ml of the renin granule fraction between the control and experimental rats. When the granule fraction of vitamin E-deficient rats was incubated

Table I. Effect of Ascorbic Acid on Renin Release and Lipid Peroxide Formation in the Renin Granule Fraction

Experimental group	Renin release (%)			Lipid peroxide formation (nmol of MDA/mg protein)		
	Ascorbic acid			Ascorbic acid		
	0 μM	10 μM	20 μM	0 μM	10 μM	20 μM
Control	18.0 ± 2.27	67.6 ± 2.65[e]	78.4 ± 2.00[e]	1.37 ± 0.11	7.14 ± 0.24[e]	8.54 ± 1.37[e]
Vitamin E-deficient	31.5 ± 3.13[a]	90.8 ± 2.27[c,e]	94.8 ± 2.48[b,e]	2.07 ± 0.24[a]	8.58 ± 1.03[e]	10.1 ± 0.57[e]
TOCA supplemented	23.0 ± 2.66	55.6 ± 5.83[d]	61.0 ± 7.97[d]	1.20 ± 0.13	5.83 ± 0.69[e]	7.83 ± 1.07[e]
DPPD supplemented	21.7 ± 2.74	26.8 ± 2.29[c]	29.0 ± 4.56[c]	0.86 ± 0.18[a]	1.03 ± 0.23[c]	1.18 ± 0.23[c]

All values are means ± S.E. of four separate experiments. *a*—*c*) Values are significantly different from the control value (*a*, $p<0.05$; *b*, $p<0.01$; *c*, $p<0.001$). *d*, *e*) Values are significantly different from the value without ascorbic acid in each group (*d*, $p<0.01$; *e*, $p<0.001$).

Table II. Effect of Ferrous Ions on Renin Release and Lipid Peroxide Formation in the Renin Granule Fraction

Experimental group	Renin release (%)			Lipid peroxide formation (nmol of MDA/mg protein)		
	Ferrous ions			Ferrous ions		
	0 μM	10 μM	20 μM	0 μM	10 μM	20 μM
Control	20.2 ± 1.62	44.0 ± 1.80[e]	61.2 ± 1.22[e]	1.62 ± 0.13	8.04 ± 0.57[e]	10.0 ± 0.92[e]
Vitamin E-deficient	36.9 ± 2.55[b]	67.3 ± 2.71[c,e]	90.9 ± 3.67[c,e]	2.40 ± 0.28[a]	9.54 ± 0.98[e]	11.5 ± 1.20[e]
TOCA supplemented	24.9 ± 1.91	43.0 ± 5.73[d]	63.7 ± 4.76[e]	1.45 ± 0.20	7.14 ± 0.42[e]	9.00 ± 0.53[e]
DPPD supplemented	22.2 ± 2.40	34.8 ± 3.20[a,d]	35.6 ± 3.93[c,d]	0.98 ± 0.12[a]	3.84 ± 0.29[c,e]	4.33 ± 0.25[e]

All values are means ± S.E. of four separate experiments. *a*—*c*) Values are significantly different from the control value (*a*, $p<0.05$; *b*, $p<0.01$; *c*, $p<0.001$). *d*, *e*) Values are significantly different from the value without ferrous ions in each group (*d*, $p<0.05$; *e*, $p<0.001$).

alone at 37 °C for 30 min, approximately 30% of total renin appeared in the incubation medium. The lipid peroxide level in this fraction was also increased to about 150% of the control value (Table I). When the renin granule fraction was incubated in the presence of 10 or 20 μM ASA, the amount of renin release in vitamin E-deficient rats reached an extremely high value, *i.e.*, 90—95% of total renin was released during incubation at 37 °C for 30 min. These values of renin release due to ASA were significantly higher than the control values under the same incubation conditions. Similarly, renin release from renin granules was markedly stimulated by incubation at 37 °C in the presence of 10 or 20 μM ferrous ions in the control group (Table II). The values of renin release due to ferrous ions in vitamin E-deficient rats were significantly higher than those in control rats. Lipid peroxide formation in the renin granule fraction was also enhanced by the addition of ASA or ferrous ions to the incubation medium, and the increase of lipid peroxides in the vitamin E-deficient group appeared to be higher than in the control group (Tables I and II).

It is well known that iron components, probably inorganic iron ions, are essential for the formation of lipid peroxides in subcellular particulates. The requirement for iron appears to be specific, and no other metal ions can replace it.[12] The role of ASA is thought to be maintaining iron ions in their reduced form.[13] In the present study, the renin granules of vitamin E-deficient rats were shown to be susceptible to ASA and ferrous ions, which produced a high level of renin release with increased lipid peroxidation in the renin granule fraction. Thus, it is considered that vitamin E deficiency causes impairment of the membrane integrity of renin granules, leading to increased sensitivity of the granules to renin release by ASA and ferrous ions.

Recently, we reported[6] that the increased release of renin from the granules due to vitamin E deficiency was reversed to the control value by dietary supplementation of TOCA or DPPD. In the present study, the effects of ASA and ferrous ions on renin release from the granules were examined after supplementation of TOCA or DPPD to the vitamin E-deficient rats. As shown in Tables I and II, the increased levels of renin release and lipid peroxide formation due to ASA and ferrous ions were reversed to the control values by TOCA supplementation, and decreased to below the control values by DPPD supplementation. Since DPPD is well known to have a strong antioxidative effect on lipid peroxidation in subcellular particulates, the action of this agent can be interpreted in terms of diminished auto-oxidation of renin granule membranes during incubation at 37 °C.

The findings in the present study indicate that vitamin E functions in the maintenance of membrane integrity of renin granules by inhibiting lipid peroxidation.

References

1) A. L. Tappel, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **32**, 1870 (1973).
2) J. A. Lucy, *Ann. N. Y. Acad. Sci.*, **203**, 4 (1972).
3) A. L. Tappel, *Ann. N. Y. Acad. Sci.*, **203**, 12 (1972).
4) Y. Matsumura, T. Shimizu, Y. Ohno, and S. Morimoto, *Chem. Pharm. Bull.*, **30**, 3742 (1982).
5) Y. Matsumura, Y. Ohno, N. Miyawaki, T. Shimizu, and S. Morimoto, *J. Pharm. Dyn.*, **5**, 1030 (1982).
6) S. Morimoto, Y. Matsumura, Y. Ohno, and N. Miyawaki, *J. Pharm. Dyn.*, **6**, 844 (1983).
7) S. Morimoto, K. Yamamoto, and J. Ueda, *J. Appl. Physiol.*, **33**, 306 (1972).
8) S. Morimoto, R. Abe, A. Fukuhara, K. Tanaka, and K. Yamamoto, *Am. J. Physiol.*, **237**, F367 (1979).
9) H. Ohkawa, N. Ohishi, and K. Yagi, *Anal. Biochem.*, **95**, 351 (1979).
10) O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
11) A. Bensadoun and D. Weinstein, *Anal. Biochem.*, **70**, 241 (1976).
12) E. D. Wills, *Biochem. J.*, **113**, 325 (1969).
13) A. Ottolenghi, *Arch. Biochem. Biophys.*, **79**, 355 (1959).