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Effects of vitamin E on microsomal Ca^{2+}-ATPase activity and calcium levels in streptozotocin-induced diabetic rat kidney

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Vitamin E treatment has been found to be beneficial in preventing or reducing diabetic nephropathy. Increased tissue calcium and abnormal microsomal Ca^{2+}-ATPase activity have been suggested as contributing factors in the development of diabetic nephropathy. This study was undertaken to test the hypothesis that vitamin E reduces lipid peroxidation and can prevent the abnormalities in microsomal Ca^{2+}-ATPase activity and calcium levels in kidney of streptozotocin (STZ)-induced diabetic rats. Male rats were rendered diabetic by a single STZ injection (55 mg kg^{-1} i.p.). After diabetes was verified, diabetic and age-matched control rats were untreated or treated with vitamin E (400–500 IU kg^{-1} day^{-1}, orally) for 10 weeks. Ca^{2+}-ATPase activity and lipid peroxidation (MDA) were determined spectrophotometrically. Blood glucose levels increased approximately five-fold (>500 mg dl^{-1}) in untreated-diabetic rats but decreased to 340 ± 27 mg dl^{-1} in the vitamin E treated-diabetic group. Kidney MDA levels did not significantly change in the diabetic state. However, vitamin E treatment markedly inhibited MDA levels in both control and diabetic animals. Ca^{2+}-ATPase activity was 0.483 ± 0.008 U l^{-1} in the control group and significantly increased to 0.754 ± 0.010 U l^{-1} in the STZ-diabetic group ($p < 0.001$). Vitamin E treatment completely prevented the diabetes-induced increase in Ca^{2+}-ATPase activity (0.307 ± 0.025 U l^{-1}, $p < 0.001$) and also reduced the enzyme activity in normal control rats. STZ-diabetes resulted in approximately two-fold increase in total calcium content of kidney. Vitamin E treatment led to a significant reduction in kidney calcium levels of both control and diabetic animals ($p < 0.001$). Thus, vitamin E treatment can lower blood glucose and lipid peroxidation, which in turn prevents the abnormalities in kidney calcium metabolism of diabetic rats. This study describes a potential biochemical mechanism by which vitamin E supplementation may delay or inhibit the development of cellular damage and nephropathy in diabetes. Copyright © 2003 John Wiley & Sons, Ltd.

KEY WORDS — Streptozotocin diabetes; Ca^{2+}-ATPase activity; kidney; vitamin E

INTRODUCTION

A number of studies have reported the existence of vitamin E deficiency in diabetic patients[1,2] and experimental diabetes.[3] Vitamin E treatment has been found to be beneficial in preventing or reducing some complications of diabetes mellitus including atherosclerosis,[4] macroangiopathy,[5] neuropathy[6] and nephropathy.[7,8] Previous studies in diabetic patients and rats have reported that vitamin E supplementation improves beta-cell function, increases plasma insulin[1] and can lower blood glucose and glycated haemoglobin levels.[1,9,10] Vitamin E supplementation can decrease or inhibit lipid peroxidation and overcomes abnormalities in endogenous antioxidant defence systems in diabetic patients[10,11] and animals.[5,8,12,13] The protective effect of vitamin E administration against oxidative damage of diabetes has been demonstrated in various cells types including blood, liver, vessel, heart and kidney.[3,5,8,9,11–13]

Studies of experimentally-induced diabetic animals and patients with diabetes have shown that intracellular

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calcium levels are generally increased in tissues.[14–16] These studies reveal that abnormal intracellular calcium metabolism is a common defect in diabetes. The activity of membrane Ca^{2+}-ATPase, which plays a role in fine tuning the regulation of intracellular calcium levels, is also altered in the diabetic state. Decreased Ca^{2+}-ATPase activity has been observed in heart sarcolemma from insulin-deficient diabetic rats,[16,17] and in red blood cell membranes from IDDM patients.[18] Increased Ca^{2+}-ATPase activity was observed, however, in erythrocytes[19] and kidney basolateral membranes of non-insulin-dependent diabetic animals[20] and in kidney microsomes of STZ-diabetic rats.[15]

Recently, the antioxidant vitamin E was identified as an inhibitor of the diacylglycerol-protein kinase C (DAG-PKC) pathway.[21] Although this property of vitamin E has been suggested as a possible mechanism contributing to the beneficial effects of this antioxidant in reducing vascular[5,21,22] and glomerular[23] complications of diabetes, the exact mechanism by which vitamin E exerts its beneficial effect on nephropathic symptoms of diabetes is not clear. This study was undertaken to test the hypothesis that the treatment of diabetic rats with vitamin E can prevent the abnormalities in microsomal Ca^{2+}-ATPase activity and calcium content of kidney. Lipid peroxidation in kidney was also evaluated in experimental animals.

MATERIALS AND METHODS

Induction of diabetes and treatment protocols

Male Wistar rats, body weight 250–300 g, were fed a standard rat chow diet and had access to water *ad libitum*. Diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ, 55 mg kg^{-1} body weight) to animals fasted overnight. Diabetes was verified 48 h later by measuring tail vein blood glucose, and the rats with blood glucose levels of 300 mg dl^{-1} or more were considered as diabetic. Two days after injection of STZ or vehicle, rats were divided into the following groups: (1) untreated diabetic rats ($n = 12$); (2) diabetic rats treated with vitamin E (alpha-tocopheryl acetate, 400–500 IU kg^{-1} day^{-1}, orally; $n = 11$); (3) untreated control rats ($n = 9$); (4) control rats treated with vitamin E, as given in protocol 2 ($n = 5$). The animals were treated for a period of 10 weeks beginning 48 h after either the vehicle or STZ injection. The principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were observed.

Measurement of blood glucose, lipid peroxidation and calcium concentration

Blood glucose concentrations were measured by Accutrend® GCT meter, (Roche Diagnostics).

Lipid peroxidation was assessed by measuring malondialdehyde (MDA), an end-product of fatty acid peroxidation.[24]

Tissue calcium levels were analysed by atomic absorption spectrophotometry (Perkin Elmer 2380) according to the wet-ashing procedure with nitric acid.

Membrane-enriched microsome preparation

Kidneys were cleaned, minced and then homogenized in six volumes of freshly prepared buffer A containing: 0.3 mol l^{-1} sucrose, 10 mmol l^{-1} Hepes-HCl pH 7.4 and 2 mmol l^{-1} dithiothreitol. The material was homogenized with a Teflon/glass homogenizer (Omni mixer homogeniser model 18074, Omni Int., CT, USA). The homogenate was centrifuged at 85 000 *g* for 75 min. The supernatant was discarded and the pellet was resuspended with the original volume of buffer A containing 0.6 mol l^{-1} KCl using four strokes with the pestle and centrifuged again at 85 000 *g* for 75 min. The pellet was resuspended in the original volume of buffer A. After centrifuging at 85 000 *g* for 75 min (Beckman L 7, Beckman Instruments Inc., Fullerton, California), the pellet was suspended in buffer A using four strokes with the pestle at a protein concentration of 2–7 mg ml^{-1}. The samples were frozen at −60°C until assayed. Isolation procedures were performed at + 4°C.[25]

Measurement of Ca^{2+}-ATPase activity

Ca^{2+}-ATPase activity was measured spectrophotometrically by the method of Niggli *et al*.[26] The medium contained: 120 mmol l^{-1} KCl, 60 mmol l^{-1} Hepes pH 7 (at 37°C), 1 mmol l^{-1} $MgCl_2$, 0.5 mmol l^{-1} K_2-ATP, 0.2 mmol l^{-1} NADH, 0.5 mmol l^{-1} phosphoenolpyruvate, 1 IU pyruvate kinase, 1 IU lactate dehydrogenase ml^{-1}, 500 μmol l^{-1} EGTA. After preincubation of the assay medium (total volume of 1 ml) for 4 min at 37°C, 50 μg ATPase (microsomal preparation) were added to the medium. After 2 min the reaction was started by the addition of 600 μmol l^{-1} $CaCl_2$. The ATPase activity was followed by continuously measuring the absorbance at 365 nm.

Chemicals and statistical analysis

Phosphoenolpyruvic acid, $MgCl_2$, K_2-ATP, NADH, pyruvate kinase, lactate dehydrogenase and EGTA

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were from Sigma (St. Louis, MO, USA). $CaCl_2$ and KCl were from the Merck Company. Hepes-HCl and dithiothreitol were from the Nutritional Biochemical Corporation.

Data are expressed as mean $\pm$ SEM. They were first subjected to Bartlett's test for homogeneity of variances and were given a log transformation if necessary. One-way analysis of variance was then carried out, followed by the Student–Neuman–Keuls test to estimate the significance of differences for individual between-group comparisons.

RESULTS

The body weights, blood glucose and MDA levels of animals are shown in Table 1. STZ-diabetes resulted in a significant decrease in body weight of the animals. Vitamin E supplementation did not significantly change the weight gain of control rats but markedly inhibited diabetes-induced reduction in body weight of the animals. The final blood glucose concentrations of untreated-diabetic rats were about five times higher than in normal control rats. Treatment with vitamin E produced a significant fall in blood glucose levels of diabetic animals. Nevertheless, at the end of the treatment, diabetic rats were still hyperglycaemic when compared with normal control rats. MDA levels were found to be similar between control and untreated-diabetic rats, although MDA production was significantly inhibited by the treatment with vitamin E in both control and diabetic groups.

The Ca^{2+}-ATPase activity of control animals was $0.483 \pm 0.008\,U\,l^{-1}$. A significant increase was observed in STZ-induced diabetic rat kidney at the end of 10 weeks (0.754 ± 0.010 U/L; $p < 0.001$; Figure 1). In vitamin E-supplemented groups, Ca^{2+}-ATPase activity decreased to $0.273 \pm 0.018\,U\,l^{-1}$ in control rats and to $0.307 \pm 0.025\,U\,l^{-1}$ in diabetic rats. Both values were significantly different from the corresponding values obtained from untreated control and untreated diabetic rats ($p < 0.001$).

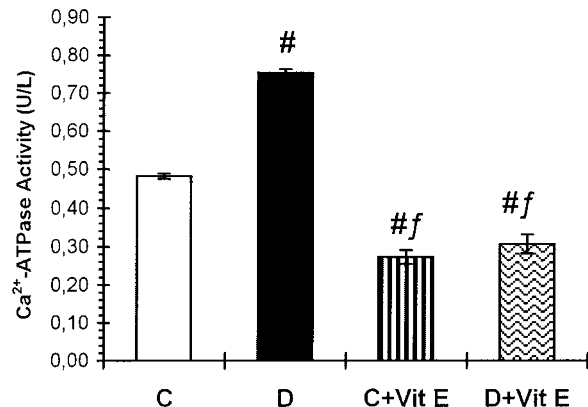

Figure 1. Microsomal Ca^{2+}-ATPase activity in kidneys obtained from control and diabetic rats untreated or treated with vitamin E. Values are the mean $\pm$ SEM. C, untreated-control ($n = 9$); D, untreated diabetic ($n = 12$); C + Vit E, control rats treated with vitamin E ($n = 5$); D + Vit E, diabetic rats treated with vitamin E ($n = 11$). $^{\#}p < 0.001$ versus untreated-control; $^{f}p < 0.001$ versus untreated-diabetic; ANOVA.

Table 1 also shows kidney calcium concentrations. As shown in this table, 10 weeks of STZ diabetes resulted in a significant increase in calcium levels. Vitamin E treatment led to marked decreases in both the control and diabetic kidney calcium levels.

DISCUSSION

Studies have shown that abnormal intracellular calcium metabolism is a common defect in both insulin-dependent and non-insulin-dependent diabetes mellitus,[14,17–20,27] and insulin-deficient diabetes appears to be associated with calcium overload in tissues.[14–16] In the present study, Ca^{2+}-ATPase activity

Table 1. Body weight, blood glucose, kidney MDA and calcium levels of non-diabetic control and 10-week STZ diabetic rats untreated or treated with vitamin E

	Non-diabetic rats		Diabetic rats	
	Control ($n = 9$)	+ Vitamin E ($n = 5$)	Control ($n = 12$)	+ Vitamin E ($n = 11$)
Final body weight (g)	312 ± 23	$337 \pm 19^{\dagger}$	$216 \pm 20^{*}$	$279 \pm 25^{\dagger}$
Blood glucose (mg dl^{-1})	98 ± 6.0	101 ± 5.0^{f}	$545 \pm 33^{\#}$	$340 \pm 27^{\#\ddagger}$
MDA (U mg^{-1} protein)	0.213 ± 0.013	$0.128 \pm 0.012^{\#f}$	0.216 ± 0.012	$0.149 \pm 0.006^{\#\ddagger}$
Kidney calcium (mg g^{-1} wet tissue)	0.491 ± 0.039	$0.210 \pm 0.012^{*f}$	$1.108 \pm 0.039^{\#}$	0.463 ± 0.076^{f}

$^{*}p < 0.05$; $^{\S}p < 0.01$; $^{\#}p < 0.001$ versus untreated-control.
$^{\dagger}p < 0.05$; $^{\ddagger}p < 0.01$; $^{f}p < 0.001$ versus untreated-diabetic; ANOVA. Means $\pm$ SEM.

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was found to be increased in diabetic rat kidney microsomes. This was accompanied by increased kidney calcium levels and is consistent with the results of our previous study.[15] Increased enzyme activity is probably due to higher calcium levels indicative of a compensatory response of the enzyme to high levels of the ion, since the Ca^{2+}-ATPase cation pump is essential for maintaining low physiological levels of intracellular calcium. The increase may also have been caused by other mechanisms involved in the activity of the enzyme, such as a change in the phospholipid and calmodulin content of the membrane as well as protein kinase-mediated phosphorylation. The phospholipid environment around the enzyme molecule exerts a considerable effect on its activity.[28] Workers have found that the phosphatidylinositol content of the kidney increases in diabetic rats.[29] The increase in the acidic phospholipid content of membranes has been suggested to be an important factor resulting in increased Ca^{2+}-ATPase activity in diabetic animals.[15,28] In addition to regulation by acidic phospholipids, polyunsaturated fatty acids, protein kinases A[30] and C,[31] cyclic GMP-dependent protein kinase (G-kinase)[32] can also activate the enzyme.

The increased intracellular concentration of calcium in the diabetic state may be explained by the osmotic activity of high glucose (cell shrinkage) that was demonstrated to activate G protein(s), most likely through a stretch receptor, which in turn stimulates calcium channels, thus permitting a calcium influx into the cells.[33] The increased cellular affinity for calcium was also reported to be due to changes in sarcolemmal lipid bilayer composition secondary to diabetes-induced hyperlipidaemia and the alterations in membrane phospholipids.[34]

In this study, we demonstrated that vitamin E treatment significantly prevents both kidney calcium accumulation and the enhancement in Ca^{2+}-ATPase activity in diabetic rats. Vitamin E treatment also led to a significant reduction in kidney calcium levels and Ca^{2+}-ATPase activity in normal control rats. These findings are in agreement with the results of our recent study, which shows that diabetes-induced calcium overload is effectively improved by this vitamin in liver and heart microsomes.[35] Although there is no study, to our knowledge, reporting the effect of vitamin E on kidney microsomal Ca^{2+}-ATPase and calcium content in diabetes-induced oxidative stress, in a related study, it was shown that the pretreatment of animals with vitamin E ameliorates sarcoplasmic reticulum calcium transport, intracellular calcium accumulation and heart dysfunction in a catecholamine-induced experimental model of oxidative stress.[36] Similarly, it has been demonstrated that vitamin E protects membrane-bound ATPases against hypercholesterolemic diet-induced impairments.[37] Vitamin E also protects Ca-dependent ATPase activity in a microsomal fraction of kidney cortex against heat ischaemia[38] and myocardial and retinal Na^{+}, K^{+}-ATPase and Ca^{2+}-ATPases against hyperglycemia-induced oxidative damage.[39] Vitamin E, a membrane-bound, lipid-soluble antioxidant, has been shown to protect biological membranes against injury induced by reactive oxygen species,[3,8,9,11–13] and to block the glycation of proteins by inhibiting MDA formation.[5,10,35] Interestingly, the treatment of bovine pulmonary artery smooth muscle microsomes with a reactive oxygen species, H_2O_2, has been found to increase calcium accumulation and Ca^{2+}-ATPase activity, and pretreatment of the microsomes with vitamin E prevented the H_2O_2-induced stimulation of Ca^{2+}-ATPase activity and also ATP-dependent Ca^{2+} uptake.[40] Indeed, diabetic tissues have an increased rate of H_2O_2 production as indicated previously,[41] and vitamin E inhibits H_2O_2 production effectively.[42] In our study, although kidney MDA levels were not significantly changed by diabetes, they were markedly inhibited by vitamin E treatment in both diabetic and non-diabetic control animals. This was concomitant with the parallel decrease in both kidney calcium levels and Ca^{2+}-ATPase activity in vitamin E-treated rats, tending to confirm the hypothesis that the occurrence of oxidant reactions is an important reason for impaired calcium metabolism in diabetic membranes. Indeed, oxidative stress increases DAG-PKC activity,[21] stimulates IP_3-induced Ca^{2+} release from sarcoplasmic reticulum,[43] leading to intracellular calcium accumulation in diabetes mellitus. Vitamin E has been shown to normalize DAG-PKC activation in diabetic rats.[21] This property of vitamin E has been suggested as a possible mechanism contributing to the beneficial effects of this antioxidant in reducing the vascular[5,21,22] and glomerular[23] complications of diabetes.

We have shown that experimental diabetes results in increases in kidney calcium levels and Ca^{2+}-ATPase activity. This result can, in turn, affect membrane permeability, alter signal transduction pathways and affect contractility and excitability and cellular dysfunctions such as nephropathy. Vitamin E can lower blood glucose and lipid peroxidation levels, prevent calcium accumulation and thereby protect Ca^{2+}-ATPase activity. This study provides a better understanding of the mechanism by which vitamin E may delay or inhibit the development of cellular damage and nephropathy in diabetes mellitus.

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