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Effect of propionyl-L-carnitine on oscillatory potentials in electroretinogram in streptozotocin-diabetic rats

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Abstract

The effect of propionyl-L-carnitine, an analogue of L-carnitine, and insulin on the oscillatory potentials of the electroretinogram was determined in rats with streptozotocin-induced diabetes. Propionyl-L-carnitine was administered at a daily dose of 0.5 g/kg by gavage for 4 weeks, while other rats were treated with subcutaneous injections of insulin (8–10 U/day). Both treatments shortened the peak latencies of the oscillatory potentials in the electroretinogram, which were significantly prolonged in untreated diabetic rats (O_1, O_2 and O_3, and $\Sigma(O_1 + O_2 + O_3)$) ($P < 0.0001$ vs. untreated normal rats). A significant decrease in the erythrocyte free carnitine level in diabetic rats was prevented by both treatments. Insulin produced a significant reduction of retinal glucose, sorbitol and fructose levels in diabetic rats, while propionyl-L-carnitine failed to do so. However, both treatments markedly reduced serum lipids levels in the diabetic rats. These findings provide information on the pathogenesis of diabetic retinopathy as well as suggesting the potential therapeutic value of propionyl-L-carnitine for retinopathy.

Keywords: Streptozotocin-induced diabetes, rat; Electroretinogram; Oscillatory potential; Propionyl-L-carnitine; Insulin; Carnitine; Sorbitol; Diabetic retinopathy; Serum lipid

1. Introduction

Photocoagulation and vitrectomy are well established as effective treatments for advanced diabetic retinopathy. However, no effective medical therapy has been established to prevent the development of vision-threatening retinopathy (Davis, 1992; Kohner, 1993). The pathogenesis of this complication of diabetes remains obscure, but several factors (metabolic, endocrine and haemodynamic abnormalities) may be important (Davis, 1992; Kohner, 1993). Recently, more evidence has become available that a low tissue carnitine concentration may be involved in the development of diabetic complications (Williamson et al., 1993; Ido et al., 1994). The improvement of diabetic complications by treatment with propionyl-L-carnitine has been demonstrated in both animal (Ido et al., 1994; Paulson et al., 1984; Pieper and Murray, 1987; Rodrigues et al., 1988; Lowitt et al., 1990, 1993; Hotta et al., 1991; Sima et al., 1994) and human (Greco et al., 1992) studies.

It is well accepted that the electroretinogram specifically indicates retinal function and also provides an early warning of retinal abnormalities before ophthalmoscopically visible alterations are detectable in diabetes (Yonemura et al., 1962; Algver, 1968). Electroretinogram changes in diabetes include the reduction in the b-wave amplitude and the prolongation of peak latencies of oscillatory potentials. The b-wave of the electroretinogram is known to be related to Müller cell function (Miller and Dowling, 1970; Tamai and Tanaka, 1973; Karwoski and Proenza, 1977; Fujimoto and Tomita, 1981; Newman and Odette, 1984) and the Müller cells of the retina develop damage due to diabetes before the retinal blood vessels are affected (Simonsen, 1968). Although it is not known which cell type in the inner retinal layer generates the oscillation, changes in the latencies of oscillatory potentials are more significant than those in the b-wave amplitude in rats with early diabetes (Kozak et al., 1983; Sato et al., 1984).

In the present study, rats with streptozotocin-induced diabetes were treated with a L-carnitine analogue, propionyl-L-carnitine or insulin, and the changes of the oscillatory potentials of the electroretinogram as well as the

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retinal sorbitol content and erythrocyte carnitine content were determined to investigate the pathogenesis of diabetic retinopathy and the mechanism of the preventive effect of this drug.

2. Materials and methods

2.1. Animals

Five-week-old male Wistar rats (Chubu Kagakushizai, Nagoya, Japan) weighing 190–200 g were used in these experiments. They were allowed to adapt to the animal facility for 7 days before use, being housed in a clean room at a temperature of 23 ± 1°C and humidity of 50 ± 10%, with a 12-h light-dark cycle and 12 changes of fresh air per h. They had free access to rat chow (Ca-1; Clea, Tokyo, Japan) and tap water. Diabetes was induced by the intravenous injection of streptozotocin (50 mg kg^{-1}), as in our previous studies (Hotta et al., 1985, 1995). Streptozotocin was dissolved in 3 mM citric acid buffer (pH 4.5) immediately before injection. Two weeks later, rats with a serum glucose level > 16.7 mM were randomly assigned to three groups. The diabetic control group was allowed free access to laboratory chow and water, and received physiological saline (the vehicle) orally once daily for 4 weeks. The other two groups of rats were also allowed free access to laboratory chow and water. One group was given propionyl-L-carnitine at a daily dose of 0.5 g kg^{-1} (dissolved in physiological saline) by gavage for 4 weeks, while the other was treated with once-daily subcutaneous injection of insulin (8–10 U; Novo lente insulin, Novo-Nordisk Japan, Tokyo, Japan). In addition, a group of normal rats was given oral propionyl-L-carnitine (0.5 g kg^{-1}) in the same manner as the diabetic rats, while the untreated control group was allowed free access to laboratory chow and water without any treatment.

2.2. Electroretinogram recording

The rats were adapted to darkness for at least 20 min, and then anesthetized by intraperitoneal injection (0.2 ml 100 g^{-1}) of a mixture of ketamine (50 mg ml^{-1}), xylazine (25 mg ml^{-1}) and physiological saline (10 : 1 : 11). Ketamine (Ketalar 50) was obtained from Sankyo Pharmaceutical (Tokyo, Japan) and xylazine (Seractal) was obtained from Bayer Japan (Tokyo, Japan). Electroretinogram recording was performed by the method of Segawa et al. (1988). Monocular recordings were obtained with the pupil maximally dilated by instillation of Mydrin P (Santen Pharmaceutical, Osaka, Japan). Photic stimulation was delivered from a xenon lamp (3G21-P; San-ei, Tokyo, Japan) at an intensity of 1 J with a 20-s interstimulus interval. Using a contact lens-type electrode, the electroretinogram was amplified (AVB-10 Preamplifier; Nihon-Koden, Osaka, Japan) with a time constant of 0.3 s and displayed on an oscilloscope (VC-10, Nihon-Koden). Groups of five potentials were summed using a signal averager (DAT-1100, Nihon-Koden) that also provided a recording (WX2400 X,Y-recorder; Graphtec, Tokyo, Japan) of the averaged electroretinogram. The peak latency was measured as the interval between stimulus start and the peak of the corresponding oscillatory potentials, and the latencies were designated as O_1, O_2 and O_3 in order of superimposition on the b-wave, as described previously (Segawa et al., 1988).

2.3. Determination of retinal sugars and polyols and erythrocyte carnitine

Rats were anesthetized with diethyl ether at 3–5 h after the completion of drug or vehicle administration and blood was collected from the heart into heparinized test tubes. In addition, the retinas were removed, weighed immediately and frozen at −70°C until determination of the sugar and polyol contents.

To measure sugars and polyols, the retinal tissue was first homogenized in 0.5 ml of cold 8% perchloric acid. After centrifugation at 12 000 × g for 5 min, the supernatant was neutralized with 2 N KOH. Then, the neutralized extracts were centrifuged at 3000 × g and the supernatant was used for the assay of polyols with a spectrofluorometer (model FP-777; Jasco, Tokyo, Japan). The sorbitol content was determined enzymatically by monitoring the conversion of sorbitol to fructose by sorbitol dehydrogenase using a slight modification of the method of Clements et al. (1969). The reaction mixture contained 30 mM glycine buffer (pH 9.4), 0.15 mM NAD^+, 1.2 U of sorbitol dehydrogenase, and the retinal sample in a total volume of 0.5 ml. Myo-inositol was assayed according to the enzymatic method of MacGregor and Matschinsky (1984). Glucose (Bergmeyer et al., 1974) was assayed by monitoring its conversion to glucose-6-phosphate by hexokinase and glucose-6-phosphate dehydrogenase, while fructose (Bernt and Bergmeyer, 1974) was determined by monitoring its conversion to glucose-6-phosphate by phosphoglucose isomerase and glucose-6-phosphate dehydrogenase. The reaction mixture for the glucose assay contained 50 mM Tris-HCl buffer (pH 7.5), 0.5 mM ATP, 0.5 mM $NADP^+$, 10 mM $MgCl_2$, 0.5 U of hexokinase, 0.5 U of glucose-6-phosphate dehydrogenase and the retinal sample in a total volume of 1.0 ml. For the fructose assay, 1.3 U of phosphoglucose isomerase was added to the above reaction mixture immediately after the determination of glucose.

For determination of the erythrocyte carnitine content, erythrocytes were homogenized in 1 ml of 0.1 M HEPES. After centrifugation at 3000 × g for 10 min at 4°C, the supernatant was heated at 60°C for 30 min and centrifuged again at 3000 × g for 5 min. After extraction of free

Table 1
Variation in body weight, serum lipids and serum insulin levels of normal and streptozotocin-induced diabetic rats

Group	Group code	n	Body weight (g)	Serum concentration			
				Glucose (mM)	Total cholesterol (mM)	Triglyceride (mM)	Insulin (μU ml^{-1})
Normal rats							
Untreated	NC	10	300.0 ± 3.7	6.72 ± 0.38	1.29 ± 0.04	0.87 ± 0.12	10.3 ± 0.6
PCAL-treated	NPC	8	298.8 ± 9.1	7.28 ± 0.40	1.13 ± 0.04	0.66 ± 0.04	10.2 ± 0.4
Diabetic rats							
Untreated	DC	10	207.0 ± 6.3 [a]	30.49 ± 1.28 [a]	2.21 ± 0.12 [a,d]	3.78 ± 0.48 [a,d]	5.3 ± 0.4 [b]
PCAL-treated	DPC	8	221.4 ± 9.6 [a]	30.95 ± 0.94 [a]	1.77 ± 0.11 [b,c]	2.49 ± 0.33 [a]	5.7 ± 0.4 [b]
Insulin-treated	DI	10	305.0 ± 6.9	10.12 ± 1.40	1.42 ± 0.05	1.35 ± 0.18 [b]	

PCAL, propionyl-L-carnitine. Results are means ± S.E.M. [a] $P < 0.0001$ vs. NC, NPC and DI; [b] $P < 0.05$ vs. NC and NPC; [c] $P < 0.0006$ vs. DI; [d] $P < 0.004$ vs. DPC.

carnitine by centrifugation, a radioisotope assay was performed, as described in detail elsewhere (McGarry and Foster, 1976).

2.4. Measurement of serum glucose, lipids and insulin

Blood was obtained from the heart and centrifuged at $3000 \times g$ for 10 min, after which aliquots of serum were tested as described previously (Hotta et al., 1992). Total cholesterol and triglyceride were measured by enzymatic methods (Determiner TC-S and TG-S, respectively; Kyowa Medex, Tokyo, Japan). Serum insulin was measured by radioimmunoassay (Insulin Riabeads; Dainabot, Tokyo, Japan) and serum glucose was determined with an autoanalyzer (Enzyme Electrode Analyzer As 200; Toyo Jozo, Tokyo, Japan).

2.5. Drugs and other chemicals

Streptozotocin was obtained from Wako Pure Chemical (Tokyo, Japan). Propionyl-L-carnitine was kindly synthesized for this study by ONO Pharmaceutical (Osaka, Japan). The other reagents and enzymes used in this study were purchased from Sigma Chemical (St. Louis, MO, USA) or Wako Pure Chemical.

2.6. Statistical analysis

Data are expressed as the means ± S.E.M. Differences between experimental groups were evaluated by analysis of variance and the significance of differences between groups was assessed by Scheffe's S test. A P value of less than 0.05 was taken to indicate significance.

3. Results

3.1. Body weight and serum glucose, lipid and insulin

The changes of body weight and the serum glucose, lipid and insulin levels for all groups are shown in Table 1. Body weight and the serum glucose, lipid and insulin levels were similar in both groups of normal rats. Unlike the insulin-treated diabetic rats, the other two groups of diabetic rats lost a significant amount of weight and developed hyperglycemia. Treatment with propionyl-L-carnitine had no effect on either weight loss or the severity of hyperglycemia. There were significant differences in the serum glucose level and body weight between the insulin-treated diabetic rats and the other diabetic rats, but there were no differences between the insulin-treated diabetic

Table 2
Effects of propionyl-L-carnitine (PCAL) and insulin on peak latencies of oscillatory potentials (O_1, O_2 and O_3) in normal and diabetic rats

Group	Group code	n	Peak latencies of oscillatory potential (ms)			
			O_1	O_2	O_3	$\Sigma(O_1 + O_2 + O_3)$
Normal rats						
Untreated	NC	10	26.5 ± 0.5	36.2 ± 0.5	48.0 ± 0.6	110.7 ± 1.5
PCAL-treated	NPC	8	24.9 ± 0.7	34.1 ± 0.5	46.6 ± 0.8	105.6 ± 1.7
Diabetic rats						
Untreated	DC	10	30.4 ± 0.6 [a]	38.6 ± 0.6 [a]	51.4 ± 0.5 [a]	120.4 ± 1.5 [a]
PCAL-treated	DPC	8	25.1 ± 0.5	35.9 ± 0.4	49.2 ± 0.5	110.3 ± 1.1
Insulin-treated	DI	10	24.6 ± 0.4	34.0 ± 0.6	46.8 ± 0.6	105.4 ± 1.4

Results are means ± S.E.M. [a] $P < 0.02$ vs. NC, NPC, DPC and DI.

Table 3
Effects of propionyl-L-carnitine (PCAL) and insulin on the glucose and polyol contents of the retina in normal and diabetic rats

Group	Group code	n	nmol g^{-1} wet weight			
			Glucose	Sorbitol	Fructose	Myo-inositol
Normal rats						
Untreated	NC	10	46.3 ± 17.5	27.1 ± 2.3	48.2 ± 10.2	1377.3 ± 70.8
PCAL-treated	NPC	8	25.3 ± 15.9	37.7 ± 7.4	75.5 ± 12.9	1368.9 ± 74.1
Diabetic rats						
Untreated	DC	10	2063.3 ± 89.1 [a]	1075.8 ± 100.5 [a]	962.5 ± 41.7 [a]	1218.5 ± 79.7
PCAL-treated	DPC	8	1886.6 ± 219.9 [a]	984.0 ± 85.7 [a]	961.0 ± 106.8 [a]	1244.9 ± 26.5
Insulin-treated	DI	10	780.9 ± 280.8 [b]	60.3 ± 13.3	174.5 ± 26.7	1369.8 ± 47.7

Results are means ± S.E.M. [a] $P < 0.05$ vs. NC, NPC and DI; [b] $P < 0.05$ vs. NC and NPC.

rats and the normal rats. Serum lipids (total cholesterol and triglycerides) were highest in the untreated diabetic rats, showing a significant difference from the other four groups. A significant difference in serum lipid levels was also observed between the propionyl-L-carnitine-treated diabetic rats and the insulin-treated diabetic rats. In addition, these two groups had higher lipid levels than did the normal rats, with the exception of no difference in total cholesterol between the insulin-treated diabetic rats and the untreated normal rats. A significant difference in serum insulin levels was observed between the normal rats and the diabetic rats (note that serum insulin was not determined in the insulin-treated group). Treatment with propionyl-L-carnitine had no effect on the serum insulin level.

3.2. Effect of propionyl-L-carnitine and insulin on the electroretinogram

The main abnormality observed in electroretinogram of the diabetic rats was prolongation of the peak latencies of oscillatory potentials (O_1, O_2 and O_3) (Table 2). There was a significant prolongation of the peak latency of each individual oscillatory potential (O_1, O_2 and O_3) and the summated potential ($\Sigma(O_1 + O_2 + O_3)$) in the untreated diabetic rats compared with the untreated normal rats. The most prominent change in the peak latency was detected in the oscillatory potential O_1. Treatment with propionyl-L-carnitine did not affect the latencies in the normal rats, however the prolongation of these latencies in the diabetic rats was significantly reduced and normalized by the treatment with propionyl-L-carnitine. Insulin treatment had an effect on the latencies of the oscillatory potentials similar to that of propionyl-L-carnitine treatment.

3.3. Effect of propionyl-L-carnitine and insulin on retinal sugars and polyols

The retinal sorbitol, glucose and fructose concentrations were markedly elevated in the untreated diabetic rats, while the myo-inositol content was unchanged (Table 3). Insulin therapy significantly decreased the retinal sorbitol, glucose and fructose concentrations in diabetic rats, but the myo-inositol content was again unchanged. Propionyl-L-carnitine treatment failed to reduce the elevated retinal glucose, sorbitol and fructose concentrations in diabetic rats, and also had no effect on the myo-inositol content.

3.4. Effect of propionyl-L-carnitine and insulin on erythrocyte free carnitine

The erythrocyte free carnitine content showed a marked decrease in the untreated diabetic rats compared with the untreated normal rats (Table 4). Treatment with propionyl-L-carnitine significantly increased the erythrocyte free carnitine content in both normal and diabetic rats. There were no significant differences between the untreated normal rats and the insulin-treated diabetic rats, between the untreated normal rats and the propionyl-L-carnitine-treated diabetic rats, and between the two treated diabetic groups. Erythrocyte free carnitine levels were highest in the propionyl-L-carnitine-treated normal rats, and significantly different from those in the other four groups.

3.5. Correlations between the ERG, plasma lipids and erythrocyte free carnitine

Correlations between the summed oscillatory potentials in the ERG, plasma lipid concentrations and the erythrocyte free carnitine content in untreated, propionyl-L-carni-

Table 4
Effect of propionyl-L-carnitine (PCAL) and insulin on free carnitine contents of erythrocyte in normal and diabetic rats

Group	Group code	n	Free carnitine nmol ml^{-1} packed cells
Normal rats			
Untreated	NC	10	18.7 ± 1.2
PCAL-treated	NPC	8	25.0 ± 1.9 [c]
Diabetic rats			
Untreated	DC	10	8.5 ± 0.4 [a]
PCAL-treated	DPC	8	17.0 ± 1.6 [b]
Insulin-treated	DI	10	20.4 ± 1.5

Results are means ± S.E.M. [a] $P < 0.0001$ vs. NC, NPC, DPC and DI; [b] $P < 0.002$ vs. NPC; [c] $P < 0.003$ vs. NC and DI.

Table 5
Correlations between plasma lipid concentrations, carnitine content in erythrocytes and the ERG ($\Sigma(O_1+O_2+O_3)$) in diabetic rats

		r value	P value
ERG ($\Sigma(O_1+O_2+O_3)$)	vs. Triglyceride	0.530	0.0037
	vs. Total cholesterol	0.635	0.0003
	vs. Carnitine	−0.681	0.0002
Carnitine	vs. Triglyceride	−0.479	0.0134
	vs. Total cholesterol	−0.447	0.0193

$\Sigma(O_1+O_2+O_3)$: sum of the peak lantencies of oscillatory potentials, O_1, O_2 and O_3 ($n=28$).

tine-treated and insulin-treated diabetic rats are shown in Table 5. There were significant positive correlations between plasma lipid concentrations and the ERG. The erythrocyte free carnitine content showed significant negative correlations with the ERG and plasma lipid concentrations.

4. Discussion

Treatment of diabetic rats with propionyl-L-carnitine, an analogue of L-carnitine, or insulin resulted in normalization of the electroretinogram abnormalities and an increase of the erythrocyte free carnitine level. Insulin treatment also produced a significant reduction in the retinal levels of glucose, sorbitol and fructose in diabetic rats, while propionyl-L-carnitine treatment failed to reduce these sugars. However, both propionyl-L-carnitine and insulin lowered serum lipid levels in the diabetic rats.

Lowitt et al. (1993) reported that treatment with acetyl-L-carnitine improved the electroretinogram abnormalities in diabetic rats without altering the erythrocyte sorbitol level and also produced a significant improvement of nerve conduction velocity along with the normalization of lipid levels in the sciatic nerve (Lowitt et al., 1990). Using propionyl-L-carnitine, we confirmed some of their observations in the present study. However, acetyl-L-carnitine needs to be administered parenterally, while propionyl-L-carnitine achieves a similar effect when given orally. Some reports have suggested that the improvement of electroretinogram abnormalities by propionyl-L-carnitine treatment may be based on the normalization of lipid metabolism in diabetic rats. Both propionyl-L-carnitine (Hotta et al., 1991) and L-carnitine (Rodrigues et al., 1988) have been shown to decrease the myocardial triacylglycerol content and increase the carnitine content in diabetic rats, resulting in the improvement of cardiac dysfunction. Ido et al. (1994) found that acetyl-L-carnitine treatment decreased serum triglyceride levels and increased nerve conduction velocity in diabetic rats. A similar effect of propionyl-L-carnitine treatment has also been observed (Hotta et al., 1996). Therefore, the correction of serum lipid levels by propionyl-L-carnitine treatment may have helped to improve electroretinogram abnormalities in our diabetic rats. The mechanism which mediates the lipid-lowering action of propionyl-L-carnitine is unknown. However, it can be speculated that administration of propionyl-L-carnitine would accelerate the transport of fatty acids into mitochondria, resulting in an increased fatty acid oxidation and decreased synthesis of triglycerides.

Haemodynamic abnormalities are well known as an important factor in the pathogenesis of diabetic retinopathy (Davis, 1992; Kohner, 1993). Propionyl-L-carnitine treatment of diabetic rats was shown to normalize sciatic nerve blood flow in a study using the hydrogen clearance method to assess endoneurial microcirculation, and the increase in blood flow was accompanied by improvement of nerve conduction velocity (Hotta et al., 1996). A variety of evidence suggests that propionyl-L-carnitine treatment may improve haemodynamic abnormalities. The cytoplasmic Ca^{2+} level in human endothelial cells is reduced by propionyl-L-carnitine (Van-Hinsbergh and Scheffer, 1991), producing a vasodilatory effect. A significant positive correlation is observed between the serum level of tissue plasminogen activator inhibitor and the serum triglyceride level (Hamsten et al., 1985). In patients with peripheral vascular disease, propionyl-L-carnitine increases tissue plasminogen activator synthesis and decreases the activity of plasminogen activator inhibitor (Pola et al., 1992), changes which would influence coagulation-fibrinolysis homeostasis. Greco et al. (1992) reported that propionyl-L-carnitine treatment significantly improved both the walking distance and symptoms of peripheral vascular disease in diabetic patients. Moreover, our previous study showed that niceritrol, a drug with lipid-lowering and peripheral vasodilatory actions, significantly decreased the serum triglyceride level and improved sciatic nerve blood flow in diabetic rats, along with an increase of tail nerve conduction velocity (Hotta et al., 1992). In the light of these findings, it seems that propionyl-L-carnitine treatment may improve electroretinogram abnormalities in diabetic rats by increasing retinal blood flow.

In the present study, we observed that both propionyl-L-carnitine and insulin increased the erythrocyte free carnitine level. Our previous studies have shown that propionyl-L-carnitine normalizes the free carnitine content of the myocardium (Hotta et al., 1991) and sciatic nerve (Hotta et al., 1996) in diabetic rats. Therefore, it is possible that normalization of the retinal free carnitine content as well as the change observed in erythrocytes was induced by propionyl-L-carnitine treatment of diabetic rats. It is supposed that the repletion of tissue L-carnitine would facilitate the transfer of acyl groups from acyl-CoA to L-carnitine, resulting in an increase of mitochondrial free CoA, disinhibition of pyruvate dehydrogenase, and normalization of the other metabolic imbalances associated with an increased mitochondrial acyl-CoA : CoA ratio (Williamson et al., 1992, 1993; Ido et al., 1994). In the present study, propionyl-L-carnitine may have corrected various imbalances in the retina by acting on the acyl-CoA : CoA path-

way to reduce the formation of lactate and increase the flow of acetyl groups through the Krebs cycle. This would lead to increased energy production in the retina and a shift to the more efficient aerobic oxidation of energy substrates, resulting in the improvement of electroretinogram abnormalities. Regarding the pathogenesis of vascular abnormalities in the retina (King et al., 1992) and aorta (King et al., 1992; Okumura et al., 1991), it is proposed that the accumulation of amphipathic lipid such as 1,2-diacyl-*sn*-glycerol and long-chain fatty acyl esters modulates the activity of some important enzymes (Na^+/K^+-ATPase and protein kinase C) linked to glucose- and diabetes-induced vascular dysfunction (Williamson et al., 1992, 1993; Ido et al., 1994). A recent study showed that acetyl-L-carnitine treatment of diabetic rats results in an increase of Na^+/K^+-ATPase activity and a decrease of 1,2-diacyl-*sn*-glycerol in the sciatic nerve, accompanied by improvement of nerve blood flow and nerve conduction velocity (Ido et al., 1994). Thus, the normalization of retinal free carnitine by propionyl-L-carnitine treatment may reduce the accumulation of 1,2-diacyl-*sn*-glycerol and/or long-chain fatty acyl esters as well as increase Na^+/K^+-ATPase activity and decrease protein kinase C activity, resulting in the normalization of electroretinogram abnormalities.

The mechanism of action of propionyl-L-carnitine may depend on the transformation of propionic acid, (which is present in the propionyl-L-carnitine molecule) into succinate. This would increase Krebs cycle energy substrates, as shown in Fig. 1, contributing to the improvement of electroretinogram abnormalities in diabetic rats. In the present study, insulin treatment of diabetic rats also produced a significant improvement of electroretinogram abnormalities, a reduction of serum lipid levels and normalization of the erythrocyte free carnitine content as well as the reduction of retinal glucose, sorbitol and fructose levels which propionyl-L-carnitine treatment failed to decrease. Recent evidence from The Diabetes Control and Complications Trial in patients with insulin-dependent diabetes mellitus has clearly shown that strict glycemic control by intensive therapy can reduce the incidence of diabetic retinopathy and delay its onset (The Diabetes Control and Complications Trial Research Group, 1993). Considering these facts and the above-mentioned mechanisms of vascular dysfunction, insulin may ameliorate hyperglycemic metabolic disorders and their related changes, while control of the factors promoting vascular dysfunction may have led to the improvement of electroretinogram abnormalities in our diabetic rats.

Well-known metabolic factors involved in the pathogenesis of diabetic complications include sorbitol accumulation caused by hyperglycemia-induced polyol pathway hyperactivity (The Diabetes Control and Complications

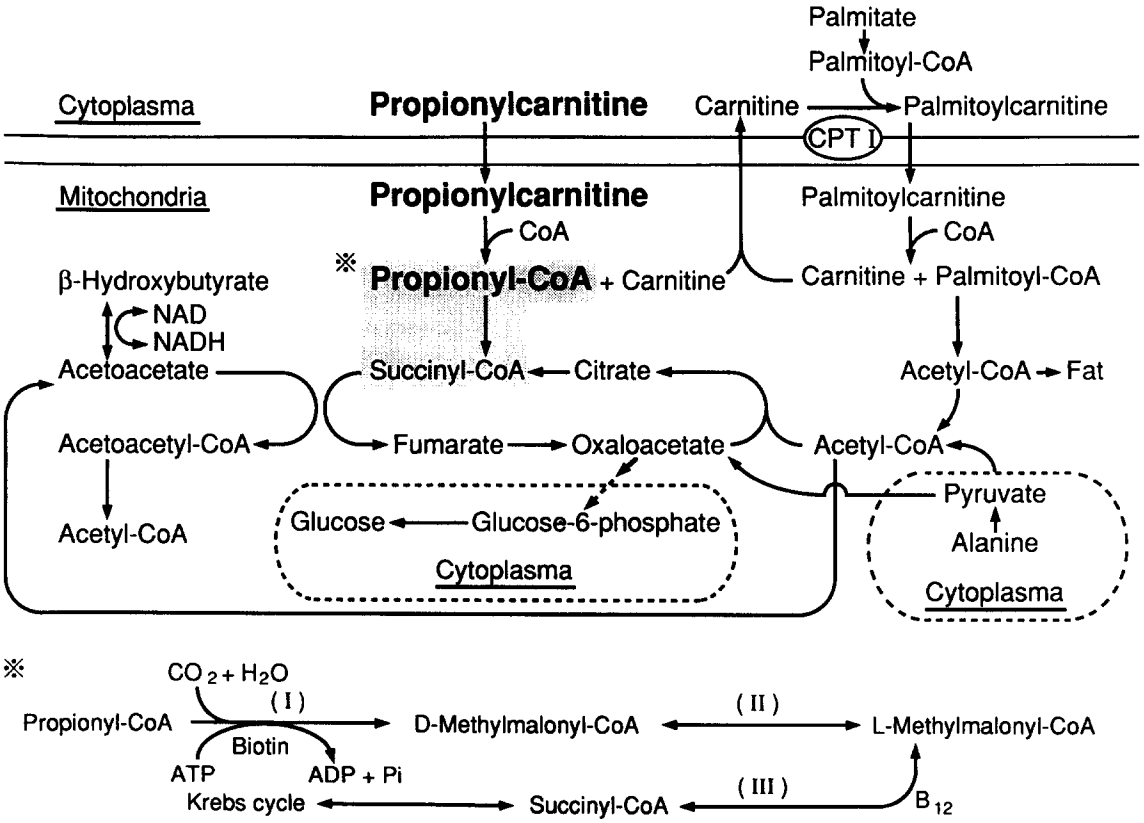

Fig. 1. Mechanism by which propionyl-L-carnitine may increase cellular energy substrates in Krebs cycle. (I), propionyl-CoA carboxylase; (II), methylmalonyl-CoA racemase, (III), methylmalonyl-CoA isomerase; CPT-I, carnitine palmitoyltransferase I.

Trial Research Group, 1993; Gabbay, 1973; Greene et al., 1988; Hotta and Sakamoto, 1990) and increased enzymatic glycation (Brownlee et al., 1988). However, propionyl-L-carnitine treatment of the diabetic rats improved electroretinogram abnormalities without having any effect on retinal sorbitol, fructose and myo-inositol levels in the present study, while insulin treatment normalized the levels of these sugars. Thus, the depletion of tissue L-carnitine may play an important role in the development of diabetic retinopathy.

In conclusion, propionyl-L-carnitine was effective to correct the electroretinogram abnormalities, increase the retinal carnitine level, and lower serum lipids levels, while the retinal content of sorbitol, fructose and myo-inositol was not affected. These findings may provide information on the pathogenetic mechanisms of diabetic retinopathy and the subsequent biochemical, functional and morphological alterations that occur as well as suggest a potential therapeutic value of propionyl-L-carnitine for retinopathy.

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