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Nerve function and blood flow in Otsuka Long-Evans Tokushima Fatty rats with sucrose feeding: effect of an anticoagulant

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Abstract

To investigate the pathogenesis of diabetic neuropathy in non-insulin-dependent diabetes mellitus, Otsuka Long-Evans Tokushima Fatty rats, an animal model of non-insulin-dependent diabetes mellitus, and non-diabetic Long-Evans Tokushima Otsuka rats were fed with or without sucrose and/or cilostazol, an anticoagulant, for 8 weeks. Sucrose-fed diabetic rats showed a delayed motor nerve conduction velocity, decreased R-R interval variability of electrocardiogram, reduced sciatic nerve blood flow, increased platelet aggregability and a decreased erythrocyte 2,3-diphosphoglycerate concentration compared with non-sucrose-fed diabetic rats and non-diabetic rats. These abnormalities were significantly prevented by treatment with cilostazol without changes in the nerve tissue levels of polyols. These findings indicate that sucrose-fed Otsuka Long-Evans Tokushima Fatty rats may be a useful animal model of neuropathy in non-insulin-dependent diabetes mellitus, and that cilostazol may prevent the development of diabetic neuropathy by modifying vascular factors.

Keywords: Cilostazol; Otsuka Long-Evans Tokushima Fatty rat; Motor nerve conduction velocity; R-R interval, coefficient of variation; Sciatic nerve blood flow; Platelet aggregation; 2,3-Diphosphoglycerate

1. Introduction

The mechanisms responsible for the diverse morphological changes affecting the nerves of humans and experimental animals with diabetes are not completely clear. A variety of hypotheses concerning the etiology of diabetic neuropathy have been proposed (Low et al., 1986; Greene et al., 1988a; Hotta and Sakamoto, 1990; Cameron and Cotter, 1993; Tomlinson et al., 1992; Vinik et al., 1992; Williamson et al., 1993; Tesfaye et al., 1994), and can be grouped into the 'metabolic', 'vascular', or 'trophic' theories.

With regard to metabolic factors, for the past 2 decades it has been suggested that hyperglycemia-induced polyol pathway hyperactivity may play an important role as an early key event in the cascade of biochemical, functional, and structural changes leading to diabetic neuropathy (Greene et al., 1988a,b; Hotta and Sakamoto, 1990; Cameron and Cotter, 1993; Tomlinson et al., 1992). However, there has also been increasing evidence recently that vascular abnormalities have a role in the pathogenesis of diabetic neuropathy (Low et al., 1986; Cameron and Cotter, 1993; Tomlinson et al., 1992; Vinik et al., 1992; Williamson et al., 1993; Tesfaye et al., 1994; Yasuda et al., 1988; Hotta et al., 1992, 1995b; Cotter et al., 1993; Tesfaye et al., 1993). Reduced peripheral nerve blood flow and consequent endoneurial hypoxia are suggested as major factors, also involving changes in blood properties, such as platelet aggregation and the 2,3-diphosphoglycerate concentration in red blood cells. Various vasodilators have been reported to have a therapeutic effect on diabetic neuropathy (Cameron and Cotter, 1993). Cilostazol (6-[4-(1-cyclohexyl-1*H*-tetrazol-5yl)butoxy]-3,4-dihydro-2(1*H*)-quinolinone) is a potent phosphodiesterase inhibitor. It blocks platelet aggregation and is a vasodilator of cerebral and femoral arteries (Kawamura et

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al., 1985; Okuda et al., 1993), and has been suggested to be of therapeutic value for diabetic neuropathy (Shindo et al., 1993; Kihara et al., 1995).

Most of the experimental animal models used for studying diabetic neuropathy involve insulin-dependent diabetes mellitus, with typical ones being rats with streptozotocin-induced diabetes and Bio-Breeding Wistar rats (Dyck et al., 1987). In contrast, there have been far fewer studies using animal models of non-insulin-dependent diabetes mellitus. While Otsuka Long-Evans Tokushima Fatty (OLETF) rats were established by Kawano et al. (1992) as an animal model of human non-insulin-dependent diabetes mellitus, which develops kidney changes similar to those of diabetic nephropathy in humans, diabetic neuropathy in this model has not yet been reported.

The present study was conducted to establish a new animal model of diabetic neuropathy and to investigate the mechanism of the therapeutic effect of cilostazol on this neuropathy. OLETF rats and Long-Evans Tokushima Otsuka (LETO) rats as non-diabetic controls were treated with sucrose and/or cilostazol for 8 weeks, and the changes in nerve function and sciatic nerve blood flow were determined. In addition, free sugar and polyol levels in the sciatic nerve, platelet aggregation, and 2,3-diphosphoglycerate concentration in red blood cells were also determined.

2. Materials and methods

2.1. Animals

5-week-old male OLETF and LETO rats (Tokushima Research Institute, Otsuka Pharmaceutical, Tokushima, Japan), weighing 130–140 and 120–130 g, respectively, were used in this study. They were housed in the animal facility for 25 weeks before use, being kept in a clean room at a temperature of 23 ± 1°C and a humidity of 50 ± 10%, with a 12-h light-dark cycle and 12 changes of fresh air per hour. They were allowed free access to rat chow (CA-1; Clea, Tokyo, Japan) and tap water. After 25 weeks, OLETF rats were randomly divided into 4 groups and LETO rats into 2 groups, as shown in Fig. 1. Two groups of OLETF rats were allowed free access to laboratory chow and plain water, and the other 2 groups were allowed free access to laboratory chow and water containing 30% sucrose (Katayama Chemical, Tokyo, Japan) for 8 weeks. One of the control groups and 1 of the sucrose-fed groups were fed with laboratory chow containing 0.03% cilostazol for 8 weeks. One group of LETO rats (the non-diabetic control) was allowed free access to laboratory chow and water containing 30% sucrose in the same manner as the sucrose-fed OLETF rats, while an untreated non-diabetic control group of LETO rats was allowed free access to laboratory chow and plain water.

2.2. Measurement of motor nerve conduction velocity, sciatic nerve blood flow, and coefficient of variation of R-R interval on the electrocardiogram

Motor nerve conduction velocity was measured in the most rapidly conducting fibers of the tail nerve supplying the segmental muscle by the method of Miyoshi and Goto (1973), as described from our previous studies (Hotta et al., 1985, 1992, 1995a,b). After the intraperitoneal injection of sodium pentobarbital (30–40 mg/kg), the rats were set on a heated pad in a room maintained at 25°C and the

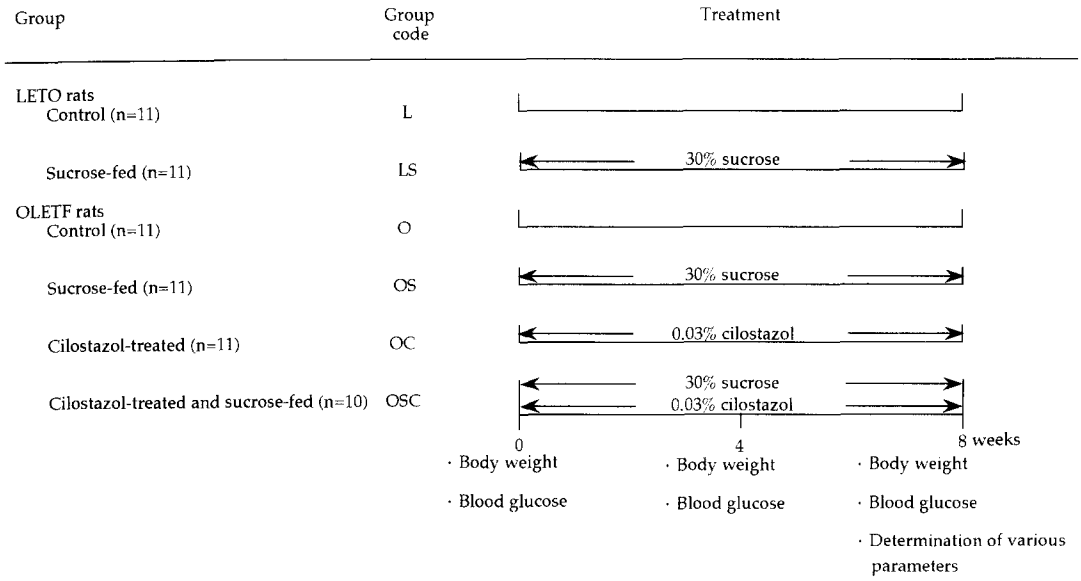

Fig. 1. Design of the present study. 30-week-old male LETO and OLETF rats were divided into 2 and 4 groups, respectively. Sucrose-fed LETO and OLETF rats received drinking water containing 30% sucrose for 8 weeks. Control LETO and OLETF rats received water without sucrose. Cilostazol-treated LETO and OLETF rats were allowed free access to laboratory chow containing 0.03% cilostazol for 8 weeks.

rectal temperature was kept constant at 37°C. Motor nerve conduction velocity was determined with a Neuropak NEM-3102 (Nihon-Koden, Osaka, Japan) at 8 weeks after the initiation of treatment. Briefly, the tail of the rat was placed in a liquid paraffin bath that was maintained at 37°C by means of a thermostat so as to ensure a constant subcutaneous temperature of the tail. The tail nerve was stimulated at 2 points: the first was 1 cm from the anus, and the second was 5 cm from the first point. A coaxial needle electrode was inserted into the segmental muscle of the tail, 4 cm from the second stimulus point. The muscle action potential induced by the 2-point stimulation of the longitudinal nerve trunk of the tail was recorded, and conduction velocity was calculated by dividing the distance between the 2 stimulus points by the latency difference.

Under anesthesia with sodium pentobarbital, sciatic nerve blood flow was measured with an electrolytic tissue blood flow meter (RBA-2; Biochemical Science, Kanazawa, Japan) and recorded with an analogue recorder (BW-24; Biochemical Science), as described previously (Hotta et al., 1992, 1995a,b). After incision of the back of the thigh and exposure of the sciatic nerve, the tip of a needle electrode (BENS 200-30; Biochemical Science) was inserted into the nerve at about 5 mm proximal to the bifurcation of the tibial and sural nerve and was advanced for about 10 mm. A reference electrode was placed in the subcutaneous tissue of the thigh. The hydrogen generated by electrolysis with 5 μA DC current for 20 min at the incision site was analyzed from the disappearance curve obtained during a constant time interval. The electrode was constructed from a Teflon-coated platinum-iridium wire (200 μm diameter). The position of the electrode was kept constant throughout the measurement because slight variations in the disappearance curves were noted that depended on the direction of insertion into the nerve. Sciatic nerve blood flow was measured at a constant room temperature in the same room as used for the determination of motor nerve conduction velocity, and sciatic nerve blood flow was calculated according to the equation of Koshu et al. (1982). Although blood pressure was not monitored during this experiment, there were no significant differences in blood pressure between each experimental group before and after anesthesia in the preliminary experiment.

The coefficient of variation of the R-R interval on the electrocardiogram was measured with a Labo-System ZS-501 (Fukuda ME, Tokyo, Japan) under light anesthesia with 25 ml of diethyl ether (Katayama Chemical) in order to avoid the influence of excessive anesthesia (Yamada et al., 1993). The rat was fixed to the instrument after the induction of anesthesia and was again anesthetized with diethyl ether for 10–15 s just before commencing electrocardiography. The electrocardiogram was recorded continuously and the data obtained in a 1-min period just before awakening (after which the heart rate exceeded 350 beats/min) were used for the determination of the coefficient of variation of the R-R interval on the electrocardiogram.

2.3. Determination of nerve tissue sugar and polyol levels

Under diethyl ether anesthesia, the sciatic nerve was removed at 3–5 h after the last meal containing cilostazol, weighed immediately, and frozen at −70°C until assay. Sugar and polyol levels in nerve tissue were measured by gas-liquid chromatography with a modification of the method of Mizuno et al. (1992). The sciatic nerve was homogenized in 1 ml of 0.2 N ZnSO4 containing an internal standard (0.05 mmol α-methyl-D-mannoside), the mixture was heated at 50°C for 10 min, and deproteinization was completed by the addition of 1 ml of 0.2 N Ba(OH)2. After centrifugation at 3000 rpm for 10 min at 4°C, the supernatant was evaporated and dried to a residue, which was silylated with 0.1 ml of *N*-trimethysilylimidazole (GL-Science, Tokyo, Japan) at 37°C for 1 h. The trimethylsilyl ethers thus formed were assayed with a gas chromatograph (GC-17A; Shimazu, Kyoto, Japan).

2.4. Assessment of platelet aggregation

Before the nerve samples were taken, blood was collected from the abdominal aorta and 4.5 ml was mixed with 1.0 ml of 3.8% trisodium citrate. Then, a platelet suspension was prepared according to the method of Ardlie et al. (1970) with minor modifications. Briefly, the citrated blood was centrifuged at 120 rpm for 10 min at room temperature. The upper portion of the supernatant was taken as platelet-rich plasma and re-centrifuged at 1100 rpm for 10 min at room temperature, after which the resultant platelet pellet was suspended in modified Tyrode's balanced salt solution (pH 7.35) containing 0.35% bovine serum albumin without calcium and magnesium. The platelet concentration was measured with a Celltac MEK-5108 (Nihon-Koden) and was adjusted to 300000/mm^3 with modified Tyrode's balanced salt solution. Then, 100 μl of this platelet suspension was placed in an NBS Hematracer 601 (Niko-Bioscience, Tokyo, Japan) and 2.0 μM ADP-induced platelet aggregation was measured by turbidimetry with constant stirring at 1000 rpm. ADP was dissolved in modified Tyrode's balanced salt solution containing calcium and magnesium. Deionized and distilled water was used as a substitute for platelet-poor plasma and the largest percentage difference of light transmittance between water and the platelet sample was calculated to determine platelet aggregation.

2.5. Measurement of 2,3-diphosphoglycerate concentration in red blood cells

After blood was obtained from the abdominal aorta, it was treated with 0.6 mM $HClO_3$ to precipitate protein and then centrifuged at 3000 rpm for 10 min. The supernatant

was neutralized with 2.5 mM KCO3 and again centrifuged at 3000 rpm for 10 min. The final supernatant was subjected to enzymatic analysis for 2,3-diphosphoglycerate using a 2,3-diphosphoglycerate UV test kit (Boehringer Mannheim, Mannheim, Germany). The hematocrit was measured simultaneously using microhematocrit tubes centrifuged at 15000 rpm for 5 min, and the 2,3-diphosphoglycerate concentration was expressed in μmol/ml of red blood cells as described previously (Hotta et al., 1995b).

2.6. Measurement of serum glucose, triglycerides, and insulin

Blood was obtained from the abdominal aorta in the 24-h-fasted state and centrifuged at 3000 rpm for 10 min, after which aliquots of serum were tested as described previously (Hotta et al., 1992, 1995a,b). Triglycerides were measured by an enzymatic method (Determiner TG-S; Kyowa Medex, Tokyo, Japan). Serum insulin was measured with a radioimmunoassay (Insulin Riabeads; Dinabot, Tokyo, Japan) and serum glucose was determined with an autoanalyzer (Enzyme Electrode Analyzer AS 200; Toyo Jozo, Tokyo, Japan).

2.7. Drugs and other chemicals

Cilostazol was kindly provided by Otsuka Pharmaceutical (Tokushima, Japan). The other reagents and enzymes used in this study were purchased from Sigma Chemical (St. Louis, MO, USA) or Wako Pure Chemical (Tokyo, Japan).

2.8. Statistical analysis

The results 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 with Scheffé's *S*-test. A P value of less than 0.05 was taken to indicate significance.

3. Results

3.1. Body weight, serum glucose, serum triglycerides, and serum insulin

The changes of body weight as well as the serum glucose, triglycerides, and insulin levels for all groups are shown in Table 1. At the beginning of the experiment, OLETF rats were heavier than LETO rats. After administration of sucrose for 8 weeks, however, the OLETF rats showed a significant weight loss while the LETO rats showed a significant weight gain. The serum glucose level showed no significant differences between the groups at the start of the experiment, but sucrose administration caused a significant elevation of serum glucose in OLETF rats. A marked increase in serum triglycerides was observed in all of the OLETF groups as compared with LETO rats, but there was no significant difference between the OLETF groups. Serum insulin levels showed no significant differences between any of the groups. Treatment with cilostazol had no effect on any of these parameters.

3.2. Effects of sucrose and cilostazol on motor nerve conduction velocity, coefficient of variation of R-R interval on the electrocardiogram, and sciatic nerve blood flow

Control OLETF rats showed a slight reduction of motor nerve conduction velocity, but the difference was not significant compared with that in control LETO rats (41.9 ± 0.9 vs. 44.1 ± 0.7 m/s) (Fig. 2A). Sucrose administration for 8 weeks significantly reduced motor nerve conduction velocity in OLETF rats (31.4 ± 0.6 m/s, $P < 0.05$) compared with that in control OLETF rats, but had no effect in LETO rats (42.8 ± 0.8 m/s). Treatment with cilostazol for 8 weeks significantly prevented the delayed motor nerve conduction velocity in sucrose-fed OLETF rats (40.6 ± 0.7 m/s, $P < 0.05$ vs. sucrose-fed OLETF rats), but had no effect in sucrose-untreated OLETF rats (41.4 ± 0.5 m/s).

Table 1
Body weight, serum glucose, serum triglycerides, and serum insulin levels in LETO and OLETF rats

Group	Group code	Diet	Baseline		8 weeks			
			Body weight (g)	Glucose (mmol/l)	Body weight (g)	Glucose (mmol/l)	Triglycerides (mmol/l)	Insulin (ng/ml)
LETO rats								
Control	L	Laboratory chow ($n = 11$)	482.8 ± 4.7	6.69 ± 0.11	518.2 ± 5.5	6.86 ± 0.29	0.83 ± 0.07	2.38 ± 0.39
Sucrose-fed	LS	Sucrose ($n = 11$)	487.3 ± 7.1	6.68 ± 0.06	540.6 ± 7.3	6.83 ± 0.26	1.13 ± 0.10	2.43 ± 0.48
OLETF rats								
Control	O	Laboratory chow ($n = 11$)	690.0 ± 9.0 [a]	8.21 ± 0.40	720.0 ± 9.7 [a]	7.86 ± 0.26	2.93 ± 0.24 [a]	2.37 ± 1.04
Sucrose-fed	OS	Sucrose ($n = 11$)	692.7 ± 9.4 [a]	7.99 ± 0.56	579.1 ± 11.9 [b]	24.48 ± 3.35 [a,b]	2.87 ± 0.21 [a]	2.53 ± 0.33
Cilostazol-treated	OC	Laboratory chow ($n = 11$)	687.3 ± 8.6 [a]	7.92 ± 0.28	721.8 ± 13.9 [a]	8.08 ± 0.31	2.78 ± 0.39 [a]	2.11 ± 0.43
Cilostazol and sucrose	OSC	Sucrose ($n = 10$)	694.0 ± 7.3 [a]	8.18 ± 0.35	562.0 ± 12.5 [b]	24.10 ± 3.16 [a,b]	2.61 ± 0.12 [a]	2.42 ± 0.28

Results are the means ± S.E.M. [a] $P < 0.05$ vs. L and LS, [b] $P < 0.05$ vs. O and OC.

Table 2
Sciatic nerve free sugar and polyol levels in LETO and OLETF rats

Group	Group code	Diet	Glucose	Sorbitol	Fructose	Myo-inositol
LETO rats						
Control	L	Laboratory chow ($n = 11$)	9.11 ± 0.70	0.44 ± 0.03	2.90 ± 0.52	6.01 ± 0.25
Sucrose-fed	LS	Sucrose ($n = 11$)	10.49 ± 0.69	0.57 ± 0.06	2.68 ± 0.14	5.91 ± 0.19
OLETF rats						
Control	O	Laboratory chow ($n = 11$)	12.57 ± 0.46	0.58 ± 0.05	3.93 ± 0.69	5.70 ± 0.30
Sucrose-fed	OS	Sucrose ($n = 11$)	16.88 ± 0.76 [a]	1.96 ± 0.20 [a]	10.26 ± 0.61 [a]	3.92 ± 0.14 [a]
Cilostazol-treated	OC	Laboratory chow ($n = 11$)	13.05 ± 0.44	0.62 ± 0.08	4.44 ± 0.43	5.05 ± 0.30
Cilostazol and sucrose	OSC	Sucrose ($n = 10$)	15.52 ± 0.51 [a]	1.81 ± 0.08 [a]	10.59 ± 0.49 [a]	3.71 ± 0.14 [a]

Results are the means ± S.E.M. in μmol/g wet weight. [a] $P < 0.05$ vs. L, LS, O and OC.

The coefficient of variation of the R-R interval on the electrocardiogram was lowest in sucrose-fed OLETF rats (1.40 ± 0.11%, $P < 0.05$) (Fig. 2B). Treatment of sucrose-fed OLETF rats with cilostazol achieved a marked prevention of this abnormality (2.77 ± 0.25%) so that it was not significantly different from the values in the other 4 groups (untreated LETO rats, 2.81 ± 0.12%; sucrose-fed LETO rats, 2.75 ± 0.22%; control OLETF rats, 3.00 ± 0.17%; cilostazol-treated OLETF rats, 2.72 ± 0.18%).

Fig. 2C shows the effect of sucrose administration and cilostazol treatment on sciatic nerve blood flow. Sucrose-fed OLETF rats had a significant decrease in sciatic nerve blood flow compared with control OLETF rats (3.5 ± 0.3 vs. 11.5 ± 0.2 ml/min/100 g, $P < 0.05$). This reduction of sciatic nerve blood flow in sucrose-fed OLETF rats was completely prevented by treatment with cilostazol (10.2 ± 0.5 ml/min/100 g, $P < 0.05$). The cilostazol-treated OLETF rats without sucrose feeding (12.2 ± 0.6 ml/min/100 g) and the 2 LETO groups (untreated control, 12.1 ± 0.5 ml/min/100 g; sucrose-fed, 12.2 ± 0.6 ml/min/100 g) also had values of sciatic nerve blood flow similar to those of the cilostazol-treated and sucrose-fed OLETF rats.

3.3. Effects of sucrose and cilostazol on nerve tissue sugar and polyol levels

The sugar and polyol concentrations in the sciatic nerve are shown in Table 2. Sucrose administration caused a marked elevation of glucose, sorbitol, and fructose concentrations and a significant reduction of the myo-inositol content in OLETF rats, but had no effect on any of these parameters in LETO rats. Cilostazol treatment failed to reduce the elevated nerve tissue glucose, sorbitol, and fructose concentrations or to increase the reduced myo-inositol content in sucrose-fed OLETF rats. Cilostazol also had no effect on these parameters in non-sucrose-fed OLETF control rats.

3.4. Effects of sucrose and cilostazol on platelet aggregation and 2,3-diphosphoglycerate concentration in red blood cells

The effects of sucrose administration and cilostazol treatment on platelet aggregation and 2,3-diphosphoglycerate concentration in red blood cells are shown in Table 3. Sucrose administration caused a significant elevation of platelet aggregation and a marked reduction of 2,3-diphosphoglycerate concentration in red blood cells of OLETF rats, but these effects were not observed in LETO rats. Treatment with cilostazol significantly prevented the changes in these 2 parameters in sucrose-fed OLETF rats.

3.5. Correlations between hematological changes and nerve function or sciatic nerve blood flow

The correlations between ADP-induced platelet aggregation or the red blood cell 2,3-diphosphoglycerate con-

Table 3
Platelet aggregation and red blood cell 2,3-diphosphoglycerate in LETO and OLETF rats

Group	Group code	Diet	Platelet aggregation (%)	RBC 2,3-diphosphoglycerate (μmol/ml red blood cells)
LETO rats				
Control	L	Laboratory chow ($n = 11$)	33.50 ± 3.17	6.57 ± 0.02
Sucrose-fed	LS	Sucrose ($n = 11$)	31.00 ± 3.95	6.79 ± 0.13
OLETF rats				
Control	O	Laboratory chow ($n = 11$)	36.50 ± 4.08	6.38 ± 0.09
Sucrose-fed	OS	Sucrose ($n = 11$)	58.38 ± 1.60 [a]	5.65 ± 0.04 [a]
Cilostazol-treated	OC	Laboratory chow ($n = 11$)	38.11 ± 3.06	6.36 ± 0.16
Cilostazol and sucrose	OSC	Sucrose ($n = 10$)	38.64 ± 3.11	6.37 ± 0.05

Results are the means ± S.E.M. [a] $P < 0.05$ vs. L, LS, O, OC, and OSC.

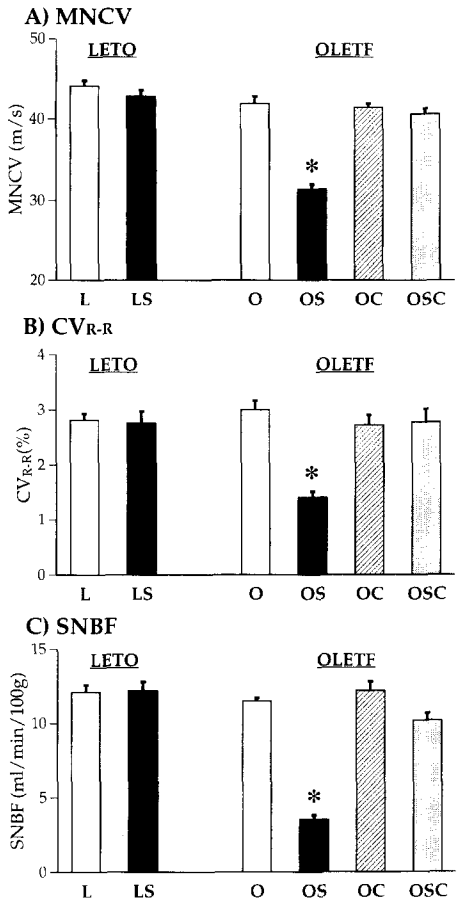

Fig. 2. Caudal motor nerve conduction velocity (A: MNCV), coefficient of variation of the R-R interval on electrocardiography (B: $CV_{R\text{-}R}$), and sciatic nerve blood flow (C: SNBF) in LETO and OLETF rats. L, untreated LETO rats; LS, sucrose-fed LETO rats; O, untreated OLETF rats; OS, sucrose-fed OLETF rats; OC, cilostazol-treated OLETF rats; OSC, cilostazol-treated and sucrose-fed OLETF rats. Results are the means ± S.E.M. * $P < 0.05$ vs. L, LS, O, OC, and OSC.

centration and nerve function or sciatic nerve blood flow were calculated for the OLETF rats (Table 4). There were significant correlations between any of the parameters of nerve function or sciatic nerve blood flow and platelet aggregation as well as the red blood cell 2,3-diphosphoglycerate concentration.

Table 4
Correlations between ADP-induced platelet aggregation or red blood cell 2,3-diphosphoglycerate level and nerve function or nerve blood flow in OLETF rats

		r	P
Platelet aggregation vs.	MNCV	0.786	0.0001
	$CV_{R\text{-}R}$	0.640	0.0001
	SNBF	0.751	0.0001
RBC 2,3-DPG vs.	MNCV	0.756	0.0001
	$CV_{R\text{-}R}$	0.530	0.0001
	SNBF	0.679	0.0001

There were 43 rats/group.
MNCV, motor nerve conduction velocity; $CV_{R\text{-}R}$, coefficient of variation of R-R interval on the electrocardiogram; SNBF, sciatic nerve blood flow; RBC 2,3-DPG, red blood cell 2,3-diphosphoglycerate.

4. Discussion

The present study used OLETF rats as an animal model of human non-insulin-dependent diabetes mellitus, in which motor nerve conduction velocity was slightly but not significantly delayed as compared with that in control LETO rats. It was clearly shown that administration of sucrose to OLETF rats for 8 weeks worsened motor nerve conduction velocity, coefficient of variation of the R-R interval on the electrocardiogram, and sciatic nerve blood flow. These changes in sucrose-fed OLETF rats were accompanied by a significant increase in platelet aggregation and a marked decrease in 2,3-diphosphoglycerate concentration in red blood cells. In addition, the anticoagulant, cilostazol significantly prevented all these changes observed in sucrose-fed OLETF rats, even though the drug had no effect on the serum levels of glucose, triglycerides, and insulin or the nerve tissue concentrations of glucose, sorbitol, fructose and myo-inositol.

It has been reported that the prevalence of diabetic neuropathy in non-insulin-dependent diabetes mellitus is equal to or even greater than that in insulin-dependent diabetes mellitus (Veglio et al., 1990). However, most experimental studies on diabetic neuropathy have involved animal models of insulin-dependent diabetes mellitus. OLETF rats were established by Kawano et al. (1992) as an animal model of human non-insulin-dependent diabetes mellitus. The post-prandial plasma glucose level of male OLETF rats becomes higher than that of LETO rats from 18 weeks of age (Kawano et al., 1992, 1994). At 24 weeks, the elevation of plasma glucose with oral glucose loading is marked in OLETF rats compared with that in LETO rats and the plasma insulin level is also higher in OLETF rats. These responses of OLETF rats to oral glucose loading became significantly more abnormal over time up to 65 weeks of age (Kawano et al., 1992, 1994). As shown in Fig. 1, there was no severe nerve dysfunction or marked reduction of sciatic nerve blood flow in control OLETF rats at 38 weeks of age and only a mild delay of motor nerve conduction velocity was observed. However, a significant delay of motor nerve conduction velocity, a marked decrease of coefficient of variation of the R-R interval on the electrocardiogram, and a significant reduction of sciatic nerve blood flow were caused by sucrose administration. Thus, the present findings suggest that the resistance of OLETF rats to the development of diabetic neuropathy may be based on a slight elevation of the serum glucose level and may not be due to a genetically determined difference in predisposition to diabetic nephropathy.

A high dietary sucrose intake has been shown to produce vascular changes (Cohen and Rosenmann, 1971; Papachristodoulou and Heath, 1977) and endoneurial edema (Powell et al., 1981) in non-diabetic rats as well as microvascular abnormalities (Cohen and Rosenmann, 1971; Papachristodoulou and Heath, 1977; Yanko et al., 1975)

and alterations of Na^+/K^+-ATPase activity (Sredy et al., 1991) in the impaired nerves of diabetic rats. However, sucrose administration led to no abnormal findings in non-diabetic LETO rats. This may reflect the shorter duration of the study than of the previous studies described above, and suggests that abnormalities in sucrose-fed OLETF rats are not caused by sucrose itself, but by hyperglycemia. In addition, a high fructose diet causes a significant delay of motor nerve conduction velocity along with a marked elevation of nerve tissue levels of sorbitol and fructose as well as a significant reduction of myo-inositol in diabetic rats (Hotta et al., 1985). Thus, it is not surprising that sucrose administration induced nerve dysfunction and reduced nerve blood flow in OLETF rats. The data in Tables 2 and 3 suggest that sucrose administration accelerated polyol pathway activity and/or altered hematological properties, resulting in the development of diabetic neuropathy. However, treatment with cilostazol significantly prevented the deficits in motor nerve conduction velocity, coefficient of variation of the R-R interval on the electrocardiogram, and sciatic nerve blood flow in sucrose-fed OLETF rats without causing any changes of nerve tissue sorbitol, fructose, and myo-inositol levels, while a significant decrease in platelet aggregation and an increase in 2,3-diphosphoglycerate concentration in red blood cells were observed. Thus, it seems unlikely that the effects of cilostazol were related to the inhibition of polyol pathway hyperactivity.

Recently, vascular factors, including blood rheological properties, have been accepted as playing a role in the pathogenesis of diabetic neuropathy (Low et al., 1986; Cameron and Cotter, 1993; Vinik et al., 1992; Williamson et al., 1993; Tesfaye et al., 1994; Yasuda et al., 1988; Hotta et al., 1992, 1995b; Cotter et al., 1993; Tesfaye et al., 1993; Dyck et al., 1987). Microcirculatory abnormalities can lead to nerve hypoxia, which in turn leads to metabolic, functional, and then structural impairment. Thus, it seems possible that hypoxia due to microvascular abnormalities, platelet hyperaggregability and a reduced 2,3-diphosphoglycerate concentration in red blood cells was the primary cause of the impairment of nerve function in our sucrose-fed OLETF rats. Cilostazol is a potent antithrombotic agent that inhibits platelet aggregation and has a vasodilator action (Kimura et al., 1985; Okuda et al., 1993). The antithrombotic action of cilostazol involves the inhibition of cyclic nucleotide phosphodiesterase type III. Its anticoagulant effect and vasodilator action have been demonstrated in both experimental animals (Kimura et al., 1985; Yasuda et al., 1985) and humans (Yasuda et al., 1985; Okuda et al., 1993). In the present study, cilostazol significantly reduced ADP-induced platelet aggregation in sucrose-fed OLETF rats, suggesting that the increase in sciatic nerve blood flow with cilostazol treatment was based on an antithrombotic and/or vasodilator effect of this agent that improved the endoneurial microcirculation and thus prevented nerve dysfunction.

The red blood cell 2,3-diphosphoglycerate level was significantly decreased in sucrose-fed OLETF rats and this decrease was prevented by cilostazol treatment (Table 3). The 2,3-diphosphoglycerate in erythrocytes has a high affinity for hemoglobin and plays an important role in regulating the binding of oxygen. A low erythrocyte 2,3-diphosphoglycerate level is observed in patients with diabetic ketoacidosis (Farber et al., 1991; Robey et al., 1987; Kowluru et al., 1989) as well as in rats with streptozotocin-induced diabetes (Hotta et al., 1995b). In our previous study (Hotta et al., 1995b), the decrease in red blood cell 2,3-diphosphoglycerate level and the reduction of sciatic nerve blood flow in diabetic rats were markedly prevented by treatment with niceritrol, a peripheral vasodilator and lipid-lowering agent, and these changes were accompanied by a marked increase in caudal nerve conduction velocity (Hotta et al., 1992). Thus, it is possible that the increase of the red blood cell 2,3-diphosphoglycerate level in cilostazol-treated OLETF rats contributed to the improvement of focal neural ischemia/hypoxia and nerve dysfunction. A report that nerve degeneration was inversely correlated with the red blood cell 2,3-diphosphoglycerate concentration in streptozotocin-induced diabetic rats with genetically determined high and normal red blood cell 2,3-diphosphoglycerate concentrations (Farber et al., 1991) indirectly supports this hypothesis.

As shown in Table 4, there were significant correlations between platelet aggregation or the red blood cell 2,3-diphosphoglycerate level and nerve function or nerve blood flow. Since cilostazol had no effect on polyol pathway hyperactivity, but significantly reduced nerve dysfunction along with platelet aggregation while increasing the red blood cell 2,3-diphosphoglycerate concentration, these findings support the possibility of a role of vascular factors, including blood rheological properties in the development of diabetic neuropathy (Cameron et al., 1991; Young et al., 1992).

In conclusion, agents, such as cilostazol which prevent rheological abnormalities, may be of therapeutic value for diabetic neuropathy (Cameron and Cotter, 1993). Although OLETF rats were originally established as an animal model of non-insulin-dependent diabetes mellitus (Kawano et al., 1992, 1994), further studies may provide information useful for the establishment of a more suitable model for the investigation of diabetic complications, including diabetic neuropathy.

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