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Effect of an aldose reductase inhibitor on abnormalities of electroretinogram and vascular factors in diabetic rats

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Abstract

The effect of an aldose reductase inhibitor, [5-(3-thienyl) tetrazol-1-yl] acetic acid (TAT), on the electroretinogram was determined in rats with streptozotocin-induced diabetes. Laboratory chow containing 0.05% TAT was given to rats for 2 months, while other diabetic rats were untreated. Groups of TAT-treated and untreated normal rats were also studied. Treatment with TAT produced significant improvement of the electroretinogram. TAT shortened the peak latencies of the b-wave oscillatory potentials, which were significantly prolonged in untreated diabetic rats ($P < 0.0001$ vs. untreated normal rats). This was accompanied by a significant decrease in the retinal sorbitol and fructose concentrations (by 46.5% and 25.7%, respectively). TAT treatment of diabetic rats also markedly reduced ADP-induced platelet aggregation and significantly increased the red blood cell 2,3-diphosphoglycerate level, accompanied by a marked reduction in sorbitol and fructose concentrations of platelet and red blood cells. There were significant correlations between the summed b-wave peak latencies and platelet aggregation or the 2,3-diphosphoglycerate level in diabetic rats. These findings suggest that an aldose reductase inhibitor, TAT, has therapeutic value for diabetic retinopathy.

Keywords: Aldose reductase inhibitor; Electroretinogram; Sorbitol; Platelet aggregation; 2,3-Diphosphoglycerate; Diabetic retinopathy

1. Introduction

Diabetic retinopathy is a serious problem and there is no effective medical therapy except maintaining good glycemic control for the prevention of vision-threatening retinopathy, although photocoagulation and vitrectomy are well established surgical therapies for advanced retinopathy (Kohner, 1993). The pathogenesis of diabetic retinopathy is extremely complex and includes factors such as metabolic, endocrine, and hemodynamic abnormalities (Kohner, 1993; Little, 1981). The hemodynamic factors contributing to retinopathy appear to have a complex etiology and, in fact, may be caused by some of the metabolic changes. During the last decade attention has been directed towards alterations in the blood and towards the role of altered blood elements in the pathogenesis of diabetic microangiopathy and retinopathy. The cause of retinal ischemia and hypoxia is probably due to a multiplicity of factors including increased platelet aggregation and reduced red blood cell 2,3-diphosphoglycerate level, which impairs the release of oxygen by hemoglobin. Thereby it can be postulated that these abnormalities contribute to the development of diabetic retinopathy. Recently, increasing evidence has become available suggesting an important role of hyperglycemia-induced polyol pathway hyperactivity in the pathogenesis of diabetic retinopathy (Robison et al., 1983; Hotta et al., 1988; Kador et al., 1990). Inhibition of polyol pathway hyperactivity improves electroretinographic abnormalities in both animals (Segawa et al., 1988; Lowitt et al., 1993) and humans (Hotta et al., 1990), and may possibly delay and/or prevent the development of diabetic retinopathy.

It is well accepted that the electroretinogram is a specific indicator of retinal function that provides an early warning of diabetic retinal abnormalities before ophthalmoscopically visible alterations are detectable (Yonemura et al., 1962; Algver, 1968). The Müller (glial) cells of the retina develop damage due to diabetes before the retinal blood vessels are affected (Simonsen, 1968) and the b-wave of the electroretinogram is known to be related to Müller cell function (Miller and Dowling, 1970; Newman and Odetta, 1984). These cells serve as the main nutritional,

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excretory, and oxygen transport system between the retinal capillaries and neurons. Thus, changes of Müller cell function may be responsible for abnormalities of the inner retinal layer related to hyperglycemia (Segawa et al., 1988). The changes of b-wave latency are more significant than those of b-wave amplitude in rats with early diabetes (Kozak et al., 1983).

In the present study, rats with streptozotocin-induced diabetes were treated with an aldose reductase inhibitor, [5-(3-thienyl) tetrazol-1-yl] acetic acid (TAT) (Hotta et al., 1995a), and the changes of b-wave latency on the electroretinogram as well as the retinal sorbitol content, red blood cell 2,3-diphosphoglycerate content, and platelet aggregation were determined to investigate the pathogenesis of diabetic retinopathy and the mechanism of the preventive effect of this agent.

2. Materials and methods

2.1. Animals

Four-week-old male Wistar rats (Chubu Kagakushizai, Nagoya, Japan) weighing 190–200 g were used in these experiments. They were acclimatized to the animal facility for 7 days before use, being housed 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 had free access to rat chow (CA-1, Clea, Tokyo, Japan) and tap water. Diabetes was induced by the intravenous injection of streptozotocin (60 mg/kg), as in our previous studies (Hotta et al., 1985, 1995a). The drug was dissolved in 3 mM citric acid buffer (pH 4.5) immediately before injection. Two weeks later, rats with a serum glucose > 16.7 mM were selected randomly and divided into two groups. The diabetic control group was allowed free access to laboratory chow and water, and the remaining rats were allowed free access to laboratory chow containing 0.05% TAT (Wakamoto Pharmaceutical, Kanagawa, Japan) and water for 2 months. In addition, a group of normal rats was allowed free access to laboratory chow containing 0.05% TAT and water in the same manner as the diabetic rats, and an untreated normal control group was allowed free access to laboratory chow and water.

2.2. Electroretinography

The rats were adapted to darkness for at least 20 min, and then anesthetized by the intraperitoneal injection of a mixture of 50 mg/ml ketamine (Ketalar 50; Sankyo Pharmaceutical, Tokyo, Japan), 25 mg/ml xylazine (Seructal; Bayer Japan, Tokyo, Japan) and physiological saline (10:1:11) at a dose of 0.2 ml/100 g body weight. Electroretinography was done by the method of Segawa et al. (1988). Monocular recordings were obtained with the pupil maximally dilated by instillation of Mydrin P (Santen Pharmaceutical Co., 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. A contact lens-type electrode was used; 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 (WX 2400 X, Y-recorder, Graphtec, Tokyo, Japan) of the averaged electroretinogram. The peak latency was measured as the interval between stimulus onset and the peak of the corresponding b-wave, and the latencies were designated as O_1, O_2 and O_3 in order of superimposition on the b-wave (Fig. 1), as described previously (Segawa et al., 1988).

2.3. Determination of retinal glucose, sorbitol and fructose

The contents of free sugars and sorbitol in the retina, platelet and red blood cell were determined by gas-liquid chromatography using a modification of the method of Mizuno et al. (1992). Under anesthesia with diethyl ether (Katayama Chemical, Tokyo, Japan), the retinas were removed and blood obtained from the abdominal aorta 3–5 h after administration of the final food containing TAT. The retinas were weighed immediately and frozen at -70°C until the glucose, sorbitol, and fructose contents were determined.

To measure sugars and sorbitol in the retina, 1 ml of 0.2 M $ZnSO_4$ containing an internal standard, 0.1 μmol α-methyl-D-mannoside, was added. To 1 ml of isolated red blood cells, 4 ml of 0.2 M $ZnSO_4$ solution containing an internal standard, 0.1 μmol of α-methyl-D-mannoside, was added. To the isolated platelets, 0.4 ml of 0.2 M $ZnSO_4$ solution containing an internal standard, 0.05 μmol of α-methyl-D-mannoside, was added. The tissues were uniformly homogenized. Each mixture solution was heated at

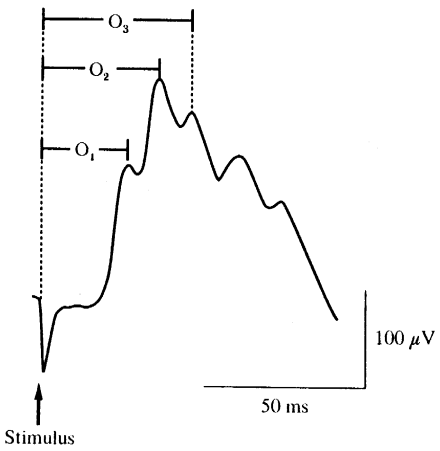

Fig. 1. Typical oscillatory potentials (designated O_1–O_3) in the b-wave of the electroretinogram in the normal rat.

50°C for 10 min, and deproteinization was completed by addition of equimolar $Ba(OH)_2$ solution. After centrifugation at 3000 rpm for 10 min at 4°C, the supernatant was evaporated and dried. The residue was silylated with 0.1 ml of *N*-trimethylsilylimidazole (GL-Sciences, Tokyo, Japan) at 37°C for 2 h. After the incubation, the trimethylsilyl ethers were assayed with a gas chromatograph (GC-17A, Shimadzu, Kyoto, Japan) on a G-100 column, 40 m × 1.2 mm ID, with a 1-μm film thickness (Chemicals Inspection and Testing Institute, Tokyo, Japan). The injector and detector temperatures were 220°C and 270°C, respectively. The initial column temperature of 190°C was held for 33 min and programmed to rise 30°C/min to 230°C. Helium was used as the carrier gas at a flow rate of 20 ml/min. Detection was made with a flame ionizer at 270°C and the area under the peak was calculated with an integrator (Chromatopack C-R7A, Shimadzu).

2.4. Assessment of platelet aggregation

Before removal of the retinal tissues, blood was collected from the abdominal aorta and 4.5 ml was mixed with 1.0 ml of 3.8% trisodium citrate. A platelet suspension was prepared according to the method of Ardlie et al. (1970) with minor modifications, and platelet aggregation activity was measured in the presence of 2.0 μM ADP (Hara et al., 1995).

2.5. Measurement of red blood cell 2,3-diphosphoglycerate

After blood was obtained from the abdominal aorta, it was treated with 0.6 mM $HClO_3$ to precipitate protein and then was centrifuged at 3000 rpm for 10 min. The supernatant was neutralized with 2.5 mM KCO_3 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 simultaneously measured with microhematocrit tubes centrifuged at 15 000 rpm for 5 min, and the 2,3-diphosphoglycerate concentration was expressed in μmol/ml of red blood cell, as described previously (Hotta et al., 1995b).

2.6. Measurement of serum glucose, lipids and insulin

Blood was obtained from the abdominal aorta and centrifuged at 3000 rpm for 10 min, after which aliquots of serum were tested as described previously (Hotta et al., 1992, 1995b).

2.7. Drugs and other chemicals

TAT was provided by Wakamoto Pharmaceutical. Streptozotocin was purchased from Wako (Tokyo, Japan). The other reagents and enzymes used in this study were purchased from Sigma (St. Louis, MO, USA) or Wako.

2.8. Statistical analysis

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

3. Results

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

The changes of body weight, and the serum glucose, lipid and insulin levels for all groups are shown in Table 1. These parameters were similar in TAT-treated and untreated normal rats. Significant weight loss, a marked increase of serum glucose and lipids (total cholesterol and triglycerides), and a significant decrease of the serum insulin level were observed in the untreated diabetic rats.

Table 1
Body weight, serum lipids and serum insulin levels in normal and diabetic rats

Group	Number	Body weight (g)	Glucose (mmol/l)	Total cholesterol (mmol/l)	Triglyceride (mmol/l)	Insulin (ng/ml)
Normal rats						
Untreated	10	448.0 ± 7.7	7.92 ± 0.47	1.58 ± 0.06	0.68 ± 0.06	2.43 ± 0.75
TAT-treated	11	455.0 ± 3.9	8.14 ± 0.44	1.69 ± 0.06	0.61 ± 0.03	2.46 ± 0.76
Diabetic rats						
Untreated	9	168.9 ± 9.8[a]	33.83 ± 1.42[a]	3.45 ± 0.39[a]	6.57 ± 1.30[a]	0.23 ± 0.24[a]
TAT-treated	10	165.0 ± 8.1[a]	33.59 ± 1.38[a]	3.70 ± 0.32[a]	6.76 ± 1.45[a]	0.23 ± 0.21[a]

Results are the means ± S.E.M. [a] $P < 0.001$ vs. normal rats.

Table 2
Peak latencies of the electroretinogram b-wave oscillatory potentials (O_1, O_2 and O_3) in normal and diabetic rats

Group	Group code	Number	O_1	O_2	O_3	$\Sigma(O_1 + O_2 + O_3)$
Normal rats						
Untreated	NC	10	26.6 ± 0.3	35.6 ± 0.3	46.8 ± 0.4	109.0 ± 0.5
TAT-treated	NT	11	27.0 ± 0.3	34.1 ± 0.4	45.8 ± 0.3	107.0 ± 0.9
Diabetic rats						
Untreated	DC	9	$31.3 \pm 0.8^{a,e}$	$41.4 \pm 1.0^{a,g}$	$55.3 \pm 1.5^{a,d}$	$127.9 \pm 3.1^{a,c}$
TAT-treated	DT	10	$28.7 \pm 0.5^{h,i}$	$38.3 \pm 0.5^{b,f}$	51.1 ± 0.5^{a}	$118.1 \pm 1.3^{b,f}$

Results are the means ± S.E.M. in milliseconds. [a]$P < 0.0001$ vs. NC and NT; [b]$P < 0.0001$ vs. NT; [c]$P < 0.0002$ vs. DT; [d]$P < 0.0005$ vs. DT; [e]$P < 0.001$ vs. DT; [f]$P < 0.002$ vs. NC; [g]$P < 0.002$ vs. DT; [h]$P < 0.004$ vs. NC; [i]$P < 0.02$ vs. NT.

Treatment with TAT had no effect on the body weight, or the serum glucose, lipid, and insulin levels in diabetic rats.

3.2. Electroretinogram changes

The untreated diabetic rats showed prolongation of the peak latencies of the b-wave potentials of the electroretinogram (Table 2). There was a significant prolongation of the peak latency of each individual oscillatory potential (O_1, O_2, and O_3) as well as that of the summed potential ($\Sigma(O_1 + O_2 + O_3)$). Prolongation of these latencies was significantly reduced by treatment with TAT.

3.3. Platelet aggregation and red blood cell 2,3-diphosphoglycerate

The effects of TAT on platelet aggregation and the red blood cell 2,3-diphosphoglycerate level are shown in Fig. 2 along with data on the summed b-wave peak latencies. There were no differences in platelet aggregation and red blood cell 2,3-diphosphoglycerate concentration between the untreated and TAT-treated normal rats ($33.2 \pm 3.2\%$ vs. $37.8 \pm 4.2\%$ and 6.4 ± 0.2 μmol/ml red blood cells vs. 6.4 ± 0.3 μmol/ml red blood cells, respectively). Diabetes significantly increased platelet aggregation to $52.4 \pm 2.2\%$ and markedly decreased the 2,3-diphosphoglycerate level to 4.3 ± 0.1 μmol/ml red blood cells. Treatment of diabetic rats with TAT significantly reversed the increase of platelet aggregation ($38.3 \pm 2.4\%$, $P < 0.03$) and the decrease of 2,3-diphosphoglycerate (5.7 ± 0.1 μmol/ml red blood cells, $P < 0.0001$).

3.4. Retinal, platelet and red blood cell sugars and sorbitol

Retinal glucose, sorbitol, and fructose concentrations were markedly elevated in the untreated diabetic rats (Table 3). Treatment with TAT significantly reduced the retinal sorbitol and fructose concentrations in diabetic rats (by 46.5% for sorbitol and by 25.7% for fructose). However, TAT treatment had no effect on the retinal glucose concentration in diabetic rats. As shown in Table 4, sorbitol and fructose levels in platelet and red blood cells were also significantly increased in the untreated diabetic rats as compared with those in the two normal groups (Table 4). Treatment with TAT significantly reduced them.

3.5. Correlations between hematological changes and b-wave potentials

The correlations between peak latencies of oscillatory potentials (O_1, O_2, O_3) and the sum of the peak latencies

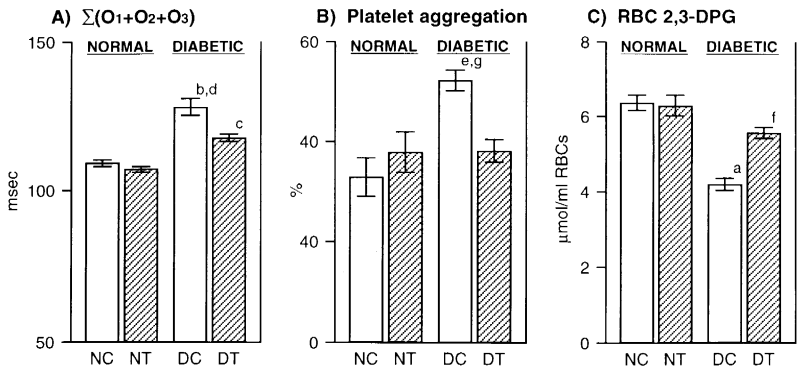

Fig. 2. Effect of TAT on the peak latency of the sum of the peak latencies of oscillatory potentials ($\Sigma(O_1 + O_2 + O_3)$), platelet aggregation, and the red blood cell (RBC) 2,3-diphosphoglycerate (DPG) levels in normal and diabetic rats. NC, untreated normal rats; NT, TAT-treated normal rats; DC, untreated diabetic rats; DT, TAT-treated diabetic rats. Results are the means ± S.E.M. [a]$P < 0.0001$ vs. NC, NT and DT; [b]$P < 0.0001$ vs. NC and NT; [c]$P < 0.0002$ vs. NC and NT; [d]$P < 0.0002$ vs. DT; [e]$P < 0.007$ vs. NC and NT; [f]$P < 0.03$ vs. NC and NT; [g]$P < 0.03$ vs. DT.

Table 3
Retinal sugar and polyol levels in normal and diabetic rats

Group	Number	Glucose	Sorbitol	Fructose
Normal rats				
Untreated	10	0.25 ± 0.18	0.03 ± 0.01	0.05 ± 0.02
TAT-treated	11	0.10 ± 0.06	0.02 ± 0.01	0.06 ± 0.01
Diabetic rats				
Untreated	9	0.93 ± 0.27[a]	0.43 ± 0.05[a,b]	0.74 ± 0.05[a,b]
TAT-treated	10	0.94 ± 0.18[a]	0.23 ± 0.03[a]	0.55 ± 0.04[a]

Results are the means ± S.E.M. in μmol/g wet weight. [a] $P < 0.0001$ vs. normal rats; [b] $P < 0.005$ vs. TAT-treated diabetic rats.

of oscillatory potentials ($\Sigma(O_1 + O_2 + O_3)$), and platelet aggregation or red blood cell 2,3-diphosphoglycerate levels were calculated in diabetic rats. As shown in Fig. 3, $\Sigma(O_1 + O_2 + O_3)$ significantly correlated with platelet aggregation and red blood cell 2,3-diphosphoglycerate levels. There were also significant correlations between peak latencies of oscillatory potentials and platelet aggregation and red blood cell 2,3-diphosphoglycerate levels (data not shown).

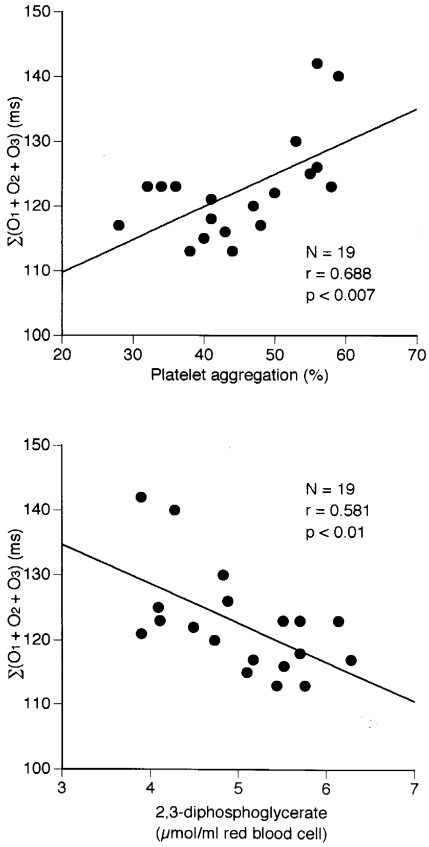

Fig. 3. Correlations between the sum of the peak latencies of oscillatory potentials ($\Sigma(O_1 + O_2 + O_3)$) and platelet aggregation or red blood cell 2,3-diphosphoglycerate levels in untreated and TAT-treated diabetic rats.

4. Discussion

The clinical validity of aldose reductase inhibitors for the treatment of diabetic retinopathy has not yet established. However, the efficacy of these agents for improving electroretinogram abnormalities has been demonstrated in diabetic rats (Kozak et al., 1983; Segawa et al., 1988; Lowitt et al., 1993) and patients with diabetes mellitus (Hotta et al., 1990). It is known that changes of b-wave latency are more significant than those of b-wave amplitude in rats with early diabetes (Kozak et al., 1983). Thus, the latency of the electroretinogram b-wave would seem to be a suitable parameter for assessing the effect of TAT on diabetic retinopathy. In the present study, diabetic rats showed a significant amelioration of electroretinogram abnormalities with TAT treatment, even though the retinal sorbitol content was only reduced by 46.5%. In other studies using the electroretinogram b-wave, the reduction in the sorbitol content by aldose reductase inhibitors has varied widely, being 95% with AND-138 (Segawa et al., 1988) and 37% with ONO-2235 (Hotta et al., 1985) in the retina, and 37% with sorbinil in erythrocytes (Lowitt et al., 1993).

It is well known that hemodynamic abnormalities play an important role in the development of diabetic retinopathy (Kohner, 1993; Little, 1981). Diabetic retinopathy leads to insufficient oxygenation of the retina (Ernest et al., 1983) and the oxygen deficit produces typical electroretinogram abnormalities (Speros and Price, 1981). Müller cells are closely related to the generation of the

Table 4
Sorbitol and fructose concentrations of platelets and red blood cells in normal and diabetic rats

Group	Group code	Number of rats	Platelet (nmol/10^9 platelets)		Red blood cells (nmol/g Hb)	
			Sorbitol	Fructose	Sorbitol	Fructose
Normal rats						
Untreated	NC	10	2.39 ± 0.37	4.69 ± 0.59	23.16 ± 2.74	133.54 ± 11.07
TAT-treated	NT	11	1.74 ± 0.91	3.50 ± 0.67	17.15 ± 2,64	110.65 ± 9.11
Diabetic rats						
Untreated	DC	9	11.92 ± 2.65[b,c,f]	11.51 ± 2.16[d,g,i]	102.57 ± 18.66[a,b,h]	300.17 ± 34.83[a,b,e]
TAT-treated	DT	10	3.14 ± 0.56	5.42 ± 0.84	49.18 ± 12.34	166.80 ± 23.04

Results are the means ± S.E.M. [a] $P < 0.0001$ vs. NC; [b] $P < 0.0001$ vs. NT; [c] $P < 0.0002$ vs. NC; [d] $P < 0.0002$ vs. NT; [e] $P < 0.004$ vs. DT; [f] $P < 0.01$ vs. DT; [g] $P < 0.02$ vs. NC; [h] $P < 0.02$ vs. DT; [i] $P < 0.05$ vs. DT.

electroretinogram b-wave and these cells serve as the main nutritional, excretory, and oxygen transport system between the retinal capillaries and neurons. Thus, alterations of Müller cell function may be responsible for abnormalities of the inner retinal layer related to hyperglycemia (Segawa et al., 1988). Attention has also been directed towards the role of altered blood elements in the pathogenesis of diabetic retinopathy (Kohner, 1993; Little, 1981). Abnormalities of blood rheologic properties in diabetes include decreased red blood cell deformability (Ernst and Matrai, 1986), increased platelet aggregation (O'Malley et al., 1975), and increased plasma viscosity (Lowe et al., 1986), and these changes may play an important role in the development of microvascular disease. As previously reported for another aldose reductase inhibitor (Takiguchi et al., 1992), TAT reversed the increase in platelet aggregation in our diabetic rats (Fig. 2). In a previous study (Hotta et al., 1995a), we found that TAT normalized sciatic nerve blood flow in diabetic rats, as assessed by hydrogen polarography, and that this improvement of the endoneurial microcirculation resulted in the normalization of nerve conduction velocity. Therefore, the reduction of platelet aggregation in diabetic rats produced by TAT would help to prevent microcirculatory damage in the retina, and thus lead to the improvement of electroretinogram abnormalities.

In addition, the red blood cell 2,3-diphosphoglycerate level in diabetic rats was significantly increased by TAT treatment (Fig. 2). 2,3-Diphosphoglycerate has a high affinity for hemoglobin and plays an important role in regulating the binding of oxygen to hemoglobin. It is well known that a low concentration of 2,3-diphosphoglycerate reduces the ability of red blood cell to release oxygen, resulting in tissue hypoxia, and a decreased red blood cell 2,3-diphosphoglycerate level is observed in patients with diabetic ketoacidosis (Alberti et al., 1972) as well as in rats with streptozotocin-induced diabetes (Hotta et al., 1995b). Many factors, including pH, phosphate, pyruvate, inosine, anemia and hypoxia, have been shown to influence the red blood cell level of 2,3-diphosphoglycerate (Belfiore, 1980). The possibility that the diabetic rats in our study had mild ketoacidosis cannot be excluded. However, the present observation that treatment with TAT, which would not alter blood pH, prevented the reduction in the red blood cell 2,3-diphosphoglycerate concentration in diabetic rats suggests that mechanisms other than acidosis are responsible for the decrease in 2,3-diphosphoglycerate. In the present study, the increased sorbitol and fructose concentrations in the red blood cells of diabetic rats were markedly decreased by TAT treatment, as was also observed in our previous study in which the increased sorbitol concentrations in the retinas and erythrocytes of diabetic rats were reduced by treatment with another aldose reductase inhibitor, ONO-2235 (Hotta et al., 1985). Therefore, it is possible that TAT improved the red blood cell 2,3-diphosphoglycerate level in diabetic rats by lowering the changes of the redox state (NAD^+ : NADH) caused by hyperglycemia-induced polyol pathway hyperactivity. The glyceraldehyde-3-phosphate dehydrogenase reaction in the cytosolic glycolytic pathway is regulated by NAD^+ : NADH. In the diabetic state, accumulation of NADH may occur due to increased sorbitol dehydrogenase activity secondary to hyperglycemia-induced activation of aldose reductase, a primary enzyme of the polyol pathway. This would induce a shift in the equilibrium of glyceraldehyde-3-phosphate and 3-phospho-glyceroyl phosphate, resulting in reduction of the red blood cell 2,3-diphosphoglycerate level. TAT apparently prevented these changes and thus raised red blood cell 2,3-diphosphoglycerate levels in the present study. Moreover, recent evidence indicates that aldose reductase inhibitors can improve endoneurial blood flow in diabetic rats by increasing NADPH and nitric oxide levels, resulting in the amelioration of diabetic neuropathy (Stevens et al., 1994). It has also been shown that red blood cell deformability is improved by the treatment of diabetic patients with sorbinil, another aldose reductase inhibitor (Robey et al., 1987). Considering our observations and the above findings, it is strongly suggested that rheological abnormalities play an important role in the deterioration of electroretinogram findings in diabetic rats. In addition, the inhibition of hyperglycemia-induced polyol pathway hyperactivity appears to partly reverse the retinal microcirculatory impairment by improving rheological abnormalities, resulting in a better oxygen supply to the Müller cells, an improvement of mitochondrial energy metabolism, and shortening of electroretinogram b-wave latencies. However, other mechanisms may also be involved, so further research is needed.

In conclusion, TAT prevented electroretinogram abnormalities in streptozotocin-induced diabetic rats, along with partial reduction of the elevated retinal sorbitol and fructose concentrations, a decrease of platelet aggregation, and correction of the decreased red blood cell 2,3-diphosphoglycerate level. These findings strongly suggest that reduced red blood cell 2,3-diphosphoglycerate level and increased platelet aggregation in addition to retinal metabolic abnormalities might play an important role in the development of diabetic retinopathy. The effect of TAT on electroretinogram abnormalities in diabetic rats might be not solely due to the reversal of hyperglycemia-induced polyol pathway hyperactivity in retinal Müller cells, and could also be related to improvement of the retinal microcirculation and the resultant increase in mitochondrial energy metabolism.

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