Polyol Metabolism of Retrograde Axonal Transport in Diabetic Rat Large Optic Nerve Fiber

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PURPOSE. The role of the polyol pathway metabolism in progressive impairment of retrograde axonal transport was evaluated in the optic nerve of rats with streptozotocin-induced diabetes.

METHODS. Rats with streptozotocin-induced diabetes received a low (3 mg/kg body weight) or high dose (10 mg/kg body weight) of oral aldose reductase inhibitor (ARI). At 1 and 3 months after induction of diabetes, Fluoro-Gold (FG, Chemicon, Temecula, CA) was injected into the dorsal lateral geniculate nucleus. Percentages of FG-labeled large, medium, and small retinal ganglion cells (RGCs) per total population were calculated in the retinas of ARI-treated diabetic, untreated diabetic, and normal control rats.

RESULTS. Mean percentages of FG-labeled large RGCs per total population were significantly decreased in nontreated diabetic rats compared with control animals at 1 month of induced diabetes. This decrease in FG labeling was not observed in both the low- and high-dose ARI-treated diabetic rats. At 3 months of induced diabetes, FG labeling of both large and medium RGCs was significantly decreased. This decrease was completely ameliorated by high-dose ARI treatment.

CONCLUSIONS. These results indicate that diabetes affects retrograde axonal transport progressively through selective impairment of RGCs and that the polyol pathway metabolism is involved in such impairment. (Invest Ophthalmol Vis Sci. 2000;41:4055–4058)

Structural analyses of peripheral nerves in humans and experimental animals with diabetes have shown that the main lesions involve axonal atrophy and axoglial dysjunction.¹ In the diabetic optic nerve, a significant reduction in mean myelinated fiber size has also been reported.²,³ Furthermore, retinal ganglion cells (RGCs) and their proximal axons show development of neuroaxonal dystrophy characterized by tubulovesicular changes, neurofilament accumulation, and lamellar membrane profile formation.² These dystrophic changes were accompanied by abnormalities in electrophysiological test results, suggesting subclinical involvement of the optic nerve in diabetes.²,³

In the nervous system, retrograde axonal transport of nerve growth factor (NGF) from target organs to neuronal cell bodies is required for normal functioning.⁴ Reduction in retrograde transport of NGF in the sciatic nerve of diabetic rats precedes the development of distal axonopathy.¹,⁵ Most studies regarding nerve dysfunction in diabetes focus on the peripheral and autonomic nervous system. Previous studies have also demonstrated retrograde axonal transport impairment and reduction in the cross-sectional size of large optic nerve fibers in diabetic rats.³,⁶ In the present study, we evaluated the impairment of retrograde axonal transport in RGCs of diabetic rats in relation to the duration of diabetes. The effect of treatment with aldose reductase inhibitor (ARI; 2-[4-{4,5,7-trifluorobenzothiazol-2-yl} methyl-3-oxo-3,4-dihydro-2H-1,4-benzothiazin-2-yl] acetic acid) on the retrograde axonal transport of the optic nerve in diabetic rats was also evaluated.

MATERIALS AND METHODS

Induction of Diabetes and ARI Treatment

Six-week-old male Wistar albino rats (Japan Clea, Osaka, Japan), each weighing approximately 200 g, were maintained in a room with an ambient temperature of 23°C. Rats were treated in accordance with the Guidelines for Animal Experimentation at Kobe University School of Medicine and in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Diabetes was induced by an intraperitoneal injection of streptozotocin (Sigma, St. Louis, MO) dissolved in 0.01 M citrate (pH 4.5) at a dosage of 70 mg/kg body weight. Age-matched normal Wistar rats served as control subjects. Rats with blood glucose levels of more than 400 mg/dL were used in the study. The diabetic rats were randomly divided into ARI-treated and ARI-untreated groups. An oral daily low (3 mg/kg body weight [BW]; n = 16) or high (10 mg/kg BW; n = 16) dose of ARI (SG-210) suspended in 0.5% carboxymethyl cellulose (CMC) solution was administered to the diabetic rats. The...
ARI-untrated \((n = 18)\) and normal control \((n = 16)\) received CMC solution without ARI for the same duration. Body weights and blood glucose levels were monitored at 2-week intervals throughout the experiment.

**Retrograde Fluorescent Labeling of RGCs**

One and 3 months after induction of diabetes, rats treated with ARI \((n = 8\) per dosage group per interval) were subjected to retrograde fluorescence labeling. The ARI-untrated \((n = 9\) per interval) and normal control \((n = 8\) per interval) groups were also subjected to retrograde fluorescence labeling at the same time intervals. Retrograde fluorescence labeling of RGCs was performed as described previously. Briefly, rats were anesthetized with an intraperitoneal injection of 5% pentobarbital sodium \((0.5 \text{ ml/kg BW})\). Glass micropipettes \((20 \mu m\) diameter) were loaded with 4% Fluoro-Gold \((\text{FG; Chemicon, Temecula, CA})\) dissolved in distilled water, and stereotaxically lowered into the dorsal lateral geniculate nuclei \((\text{dLGN})\). An arbitrary volume of dye was injected iontophoretically with a high-voltage current source and a 3-\(\mu A\) charge applied intermittently \((7\) seconds on, 7 seconds off) for 10 minutes. For adequate observation of FG-labeled RGCs, rats were killed 72 hours after FG injection. Eyes in rats showing adequate labeling of the contralateral dLGN without involvement of neighboring nuclei were enucleated. Retinas were separated from the pigmented epithelium and mounted vitreal side up on a nonfluorescent gelatin-coated glass slide. To visualize the distribution of FG-labeled RGCs in the entire retina, specimens were viewed by fluorescence microscopy with a UV filter system \((\text{main excitation wavelength: 360 nm})\), and photographed \((\text{Olympus BX51, Olympus, Tokyo, Japan})\).

**Estimation of FG-Labeled RGCs**

Photographic images of the entire retina at 1 and 3 months after induction of diabetes were saved and converted into Macintosh tagged information format \((\text{TIF})\) files and then exported to the public domain NIH Image 1.44 program for further analysis on a Macintosh computer \((\text{Apple Computer, Cupertino, CA})\). With the imaging program, soma sizes of each RGC were traced, measured, and classified into three types: large, more than 20 \(\mu m\); medium, 16 to approximately 20 \(\mu m\); and small, 15 \(\mu m\) or less. The FG-labeled RGCs were counted, measured, and classified by two reviewers \((\text{LX, HN})\) in a masked fashion. Because of the variations in the actual numbers of labeled RGCs, mean percentage values of labeled cells per total population for each RGC type were calculated and used for statistical analysis. Representative photomicrographs of control, untreated diabetic, low-dose ARI-treated diabetic, and high-dose ARI-treated diabetic retinas at 3-month intervals are shown in Figure 1.

**Statistical Analysis**

The percentage of each type of FG-labeled RGC per total population in the control, ARI-treated, and ARI-untreated diabetic groups was analyzed with Student’s \(t\)-test. \(P < 0.05\) was considered significant.

**RESULTS**

**Diabetic State**

Body weights of the ARI-treated and ARI-untreated diabetic rats were significantly reduced throughout the experimental period, whereas body weights of the control rats increased progressively \((\text{Table 1})\). Blood glucose concentration levels were significantly elevated in the ARI-treated and -untreated diabetic rats compared with control animals \((\text{Table 1})\).

**FG Labeling of RGCs at 1 Month**

Labeling of RGCs was highest 72 hours after FG injection. One month after induction of diabetes, the mean percentage of FG-labeled RGCs per total population in control subjects was calculated as 0.67% ± 0.18% for large, 8.20% ± 2.40% for medium, and 91.34% ± 2.59% for small RGCs \((\text{Fig. 2})\). In ARI-untreated diabetic rat retinas, the mean percentage of large RGCs was 0.31% ± 0.15%, representing a significant decrease compared with age-matched control retinas \((P = 0.0126)\). No significant decrease in the mean percentage of medium and small RGCs was observed between diabetic and controls retinas. The mean percentage values of FG-labeled large RGCs in both low- and high-dose ARI-treated diabetic rats was significantly improved \((P = 0.0275, P = 0.0028)\).

**FG-Labeling of RGCs at 3 Months**

The mean percentages of FG-labeled large \((P < 0.0001)\) and medium \((P = 0.0174)\) RGCs calculated from the retinal photographs were significantly decreased in diabetic rats com-
pared with control subjects (Fig. 3). High-dose ARI treatment improved the decrease in mean percentage values of FG-labeled large and medium RGCs to almost normal ($P = 0.0368$). However, treatment with low-dose ARI only partially improved the percentage values of FG-labeled large RGCs ($P = 0.0001$) and had no effect in mean percentage values of medium RGCs.

After observation by fluorescence microscopy, the retinal sections were Nissl stained and viewed again. Significant cell loss and degeneration were not observed in either ARI-treated or -untreated diabetic rat retinas (data not shown).

**DISCUSSION**

The pathophysiology of diabetic neuropathies has been investigated extensively. The polyol or sorbitol hypothesis is the most widely cited pathometabolic mechanism for diabetic neuropathy. The hypothesis relates to accumulation of sorbitol in the nerves, with a compensatory reduction in myo-inositol content, which leads to impairment in nerve function, and ultimately, to structural neuropathy. The morphologic structure of the neuron complements its capability of transmitting impulses over long distances. The anterograde axonal transport system is responsible for transporting proteins associated with axonal structure and synaptic transmitter function to the axon and its terminals. In the opposite direction is the retrograde axonal transport system, which carries neurotrophic factors that influence steady state activities in the cell body. Because neurotrophic factors are known to promote survival, maintenance, and regeneration of neurons, their role in diabetic neuropathy has been given consideration. Serum NGF levels were observed to be decreased both in humans with diabetes and experimental diabetic rats. Therefore, the disruption in normal expression of NGF under hyperglycemic states may lead to diabetic neuropathy.

The present study demonstrated a progressive deficit in the retrograde axonal transport of selective RGCs to the optic nerve. This impairment may cause reduction in expression of neurotrophic factors, which leads to downregulation in the synthesis of factors such as neurofilaments and substance P. A concomitant deficit in the anterograde axonal transport system of large myelinated optic nerve fibers is suggested to cause neuroaxonal dystrophic changes in the RGCs and the optic nerve.

ARI or myo-inositol supplementation prevents development of deficits in the orthograde axonal transport system. A concomitant deficit in the anterograde axonal transport system of large myelinated optic nerve fibers is suggested to cause neuroaxonal dystrophic changes in the RGCs and the optic nerve.

<table>
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<tr>
<th>TABLE 1. Body Weight and Blood Glucose Levels in ARI-Treated Diabetic, ARI-Untreated Diabetic, and Normal Control Rats</th>
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<tr>
<td><strong>Body Weight</strong></td>
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<tr>
<td><strong>1 Month</strong>*</td>
</tr>
<tr>
<td>Normal</td>
</tr>
<tr>
<td>Diabetes</td>
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<tr>
<td>Diabetes + low dose†</td>
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<td>Diabetes + high dose‡</td>
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Blood glucose is expressed in milligrams per decaliter.

* 1 month and 3 months represents 1 and 3 months of induced diabetes, respectively.
† 3 mg/kg body weight (in grams) oral ARI.
‡ 10 mg/kg body weight (in grams) oral ARI.
vented the early deficit in retrograde axonal transport of large RGCs. In contrast, high-dose ARI treatment prevented deficits not only in large, but also in medium, RGCs. The effect of high-dose ARI treatment on axonal transport at the 3 month interval led to amelioration of the neuroaxonal dystrophic changes previously observed in the optic nerve fibers. The present study revealed a progressive deficit in the retrograde axonal transport of selective RGCs from 1 to 3 months of induced diabetes. Twelve-months of induced diabetes may affect retrograde axonal transport in the smaller-sized RGCs and may cause a more severe form of axonal atrophy resistant to ARI treatment.

In conclusion, we demonstrated that the impairment in retrograde axonal transport of RGCs in diabetic rats is related to the duration of diabetes, with the impairment initially occurring in large RGCs and progressively affecting the medium RGCs. The impairment in retrograde axonal transport and neuroaxonal changes occurring with diabetes was prevented by ARI treatment in a dose-dependent manner. It is also interesting to note that our findings of abnormalities of large RGCs in the diabetic optic nerve are analogous to those seen not only in peripheral diabetic neuropathies, but also in glaucoma.

Acknowledgments

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References