A quantitative study of the optic nerve in diabetic mutant, Otsuka Long-Evans Tokushima Fatty (OLETF) rats

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ABSTRACT Optic nerves of the Otsuka Long-Evans Tokushima Fatty (OLETF) rat, an animal model of non-insulin dependent diabetes mellitus, were examined using quantitative stereological procedures. At 67 weeks of age, OLETF rats showed a mild hyperglycemia: their blood glucose level was $196 \pm 93$ mg/dl, significantly higher than that of non-diabetic control Long-Evans Tokushima Otsuka (LETO) rats ($110 \pm 24$ mg/dl). However, there were no differences in the cross sectional area of optic nerves (mean minimum diameter), the total number and mean diameter of both myelinated and non-myelinated fibers, or the thickness of the myelin sheath between OLETF and LETO rats. The results suggested that a mild hyperglycemia in OLETF rats could not cause any morphological changes in the optic nerve.

Key words: diabetes, optic nerve, stereology, OLETF

INTRODUCTION

Visual impairment is one of clinical signs of insulin-dependent (IDDM) and non-insulin dependent (NIDDM) diabetes mellitus in the human. Retinopathy and optic nerve abnormalities have been well studied in streptozotocin (STZ)-induced IDDM rats. These rats exhibited an increase in small caliber retinal capillaries and a reduction in the myelinated fiber size of the optic nerve (Scott et al., 1986; Inoue et al., 1998a, 1998b).

The Otsuka Long-Evans Tokushima Fatty (OLETF) rat has been established as an animal model of NIDDM (Kawano et al., 1992). This mutant is characterized by the late onset of diabetes mellitus, mild hyperglycemia, mild obesity, and insulin deficiency (Kawano et al., 1992). Multiple recessive gene defects such as cholecystokinin A receptor, *Obd1* and *Obd2*, are considered to cause the diabetes mellitus of OLETF rats (Funakoshi et al., 1994, 1995; Hirashima et al., 1996). To understand the pathogenesis of the optic nerve in NIDDM, we performed quantitative stereological procedures evaluating the cross sectional area of optic nerves (the mean minimum diameter), the total number and mean diameter of both myelinated and non-myelinated fibers, and the thickness of the myelin sheath in OLETF rats.

MATERIALS AND METHODS

Animals

Male OLETF and non-diabetic control LETO (Long-Evans Tokushima Otsuka) rats were supplied by the Tokushima Research Institute, Otsuka Pharmaceutical Co., Ltd., Japan. At 67 weeks of age, ten each of OLETF and LETO rats were deeply anesthetized with an intraperitoneal injection of sodium pentobarbital (25 μg/10 g body weight), and were perfused with 0.9% NaCl followed by 3% glutaraldehyde in 0.1 M phosphate buffer (PB) (pH 7.4). Blood samples were taken from the descending aorta before perfusion. Blood glucose level was measured by the glucose oxidase method (Cauley et al., 1959). Optic nerves were dissected out, cut at the posterior pole of the eyeball and the optic chiasma, and fixed with the same fixative overnight. Following a rinse with 0.1 M PB, the tissue was postfixed with 0.5% osmium tetroxide for 3 hours, dehydrated and embedded in Epon 812.

All animal procedures were performed according to the Guide for the Care and Use of Laboratory Animals, and were reviewed by the Institutional Animal Care and Use Committee of the University of Tokushima. All efforts were made to minimize the number of animals used and their suffering.

Sampling and stereological procedures

Sampling and subsequent stereological procedures have been described in a previous report (Fukui et al., 1991), and were briefly summarized as follows. Transverse semithin sections (about 0.5 μm) and adjacent ultrathin sections (about 70 nm) were prepared by an LKB ultramicrotome. The semithin sec-
tions were stained with toluidine blue, photographed, and the cross-sectional area of each optic nerve was measured by an MC-300 image analyzer (FLOVEL, Tokyo). The adjacent ultrathin sections were stained with uranyl acetate and lead citrate, examined with a JOEL transmission electron microscope, and photographed. At least 20 microphotographs selected by a systematic random sampling procedure (Weibel, 1972) were obtained from a single section of the optic nerve. The thickness of the myelin sheaths of myelinated fibers and the mean axonal diameter of both myelinated and non-myelinated fibers were measured by an MC-300 image analyzer. Densities of myelinated and non-myelinated fibers were separately calculated by counting their numbers within the frame, and these values together with the cross-sectional area of each optic nerve (as determined above) were used to estimate the total number of both fibers. All measurements were allowed within an oblong frame (4.5 x 2.5 μm) of each microphotograph according to the ‘forbidden’ line rule (Gundersen, 1977). Data were statistically analyzed by Student's t-test following one-way analysis of variance (ANOVA).

RESULTS

At 67 weeks of age, body weight was significantly higher in OLETF rats than in LETO rats (Table 1). OLETF rats showed a mild hyperglycemia: their blood glucose level was $196 \pm 93$ mg/dl, significantly higher than that of LETO rats ($110 \pm 24$ mg/dl).

Fig. 1 shows a light micrograph, and Fig. 2 shows an electron micrograph of transverse sections of the optic nerve in a 67-week-old OLETF rat. These micrographs were used to estimate the cross-sectional area of optic nerves, the total num-

bers and mean diameters of both myelinated and non-myelinated fibers, and the thickness of the myelin sheath.

Cross-sectional areas of optic nerves in OLETF rats were not different from those in LETO rats (Table 2). There was no difference in the total number of optic nerve fibers (myelinated plus non-myelinated fibers) nor did the number of either myelinated or non-myelinated fibers differ between OLETF and LETO rats.

Table 3 shows the mean diameters of both myelinated and

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<thead>
<tr>
<th>Table 1</th>
<th>Body weight and blood glucose level in 67-week-old OLETF and LETO rats.</th>
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<tbody>
<tr>
<td></td>
<td>OLETF (n = 10)</td>
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<tr>
<td>Body weight (g)</td>
<td>681 ± 72**</td>
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<tr>
<td>Blood glucose (mg/dl)</td>
<td>196 ± 93*</td>
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Data are given as mean ± SD. *: P < 0.02, **: P < 0.001 vs LETO rats (Student's t-test) n: number of rats examined

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Cross-sectional area and estimated number of myelinated and non-myelinated fibers of the optic nerve in OLETF and LETO rats.</th>
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<tbody>
<tr>
<td></td>
<td>OLETF (n = 10)</td>
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<td>Cross sectional area (μm²)</td>
<td>355,740 ± 27,569</td>
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<tr>
<td>Total number of optic nerve fibers</td>
<td>146,453 ± 11,920</td>
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<tr>
<td>Number of myelinated fibers</td>
<td>134,508 ± 10,766</td>
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<tr>
<td>Number of non-myelinated fibers</td>
<td>11,946 ± 3,681</td>
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Data are given as mean ± SD. n: number of rats examined

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<tr>
<th>Table 3</th>
<th>Mean diameters of myelinated and non-myelinated fibers of the optic nerve in OLETF and LETO rats.</th>
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<td></td>
<td>OLETF (n = 10)</td>
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<td>Diameter of myelinated fibers (μm)</td>
<td>1.41 ± 0.09</td>
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<tr>
<td>Axonal diameter (μm)</td>
<td>0.96 ± 0.04</td>
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<tr>
<td>Myelin sheath thickness (μm)</td>
<td>0.22 ± 0.03</td>
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<tr>
<td>Diameter of non-myelinated fibers (μm)</td>
<td>0.45 ± 0.04</td>
</tr>
</tbody>
</table>

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Fig. 1 Light micrograph of transverse section of the optic nerve in a 67-week-old OLETF rat. Bar = 100 μm.
non-myelinated fibers of the optic nerve. The mean diameters of myelinated fibers and myelinated axons were not different between OLETf and LETO rats. The thickness of the myelin sheath did not differ, nor was there any significant variation in the size distribution of myelinated axons between OLETf and LETO rats (Fig. 3). The mean diameter of non-myelinated fibers did not differ between the two groups of rats (Table 3).

**DISCUSSION**

STZ-induced IDDM rats developed optic nerve involvement characterized by a reduction in myelinated fiber size (Scott *et al.*, 1986; Inoue *et al.*, 1998a, 1998b). Retrograde or anterograde axon al transports were impaired in STZ-induced IDDM rats (Zhang *et al.*, 1998) as well as rabbits (Chihara, 1981; Chihara *et al.*, 1982). In the present study, there were no differences in any morphometrical parameter of the optic nerve between OLETf and LETO rats at 67 weeks of age, whereas the blood glucose level was significantly higher in OLETf rats than in LETO rats. In addition, retrograde axonal transport in the optic nerve was not altered in OLETf rats (Zhang *et al.*, 1998). In STZ-induced IDDM rats, the mean blood glucose level was more than 600 mg/dl (Inoue *et al.*, 1998a, 1998b), which is 3-fold higher than that in OLETf rats. Therefore, one of the reasons why OLETf rats do not develop optic nerve involvement is assumed to be the mildness of their hyperglycemia.

OLETf rats showed various retinocapillary changes such as capillary endothelial cell degeneration and capillary basement membrane thickening (Miyamura *et al.*, 1999). Similar capillary changes were observed in human diabetic patients (Stitt *et al.*, 1995) and in some diabetic animals (Wallow and Engerman, 1977; Anderson *et al.*, 1995). Thus mild hyperglycemia in OLETf rats could cause some retinocapillary changes but not optic nerve involvement.

Several reports on psychophysiological and electrophysiological examinations showed that optic nerve involvement in diabetes resembled peripheral diabetic neuropathy (Bresnik, 1986; Trick *et al.*, 1988). However, OLETf rats did not develop peripheral neuropathy (Nakamua *et al.*, 1997). When

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**Fig. 2** Electron micrograph of transverse section of the optic nerve in a 67-week-old OLETf rat. *m*: myelinated fiber; *n*: non-myelinated fiber; Bar = 1 μm.
OLETF rats were fed with sucrose for 8 weeks to induce severe hyperglycemia, they appeared to suffer dysfunction of motor and autonomic nerves (Nakamura et al., 1997). Furthermore, sorbitol accumulation and myoinositol depletion in sciatic nerves were observed in sucrose-fed OLETF rats but not in those not fed sucrose nor in sucrose-fed LETO rats (Nakamura et al., 1997). This indicated that severe hyperglycemia and increased polyol pathway activity might be necessary for the development of diabetic neuropathy. This evidence supports our assumption that the optic nerve involvement did not develop in OLETF rats because of their mild hyperglycemia.

ACKNOWLEDGMENTS

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REFERENCES


Fig. 3 Histogram for the distribution of axonal diameter of myelinated fibers of the optic nerve in 67-week-old OLETF (filled bars) and LETO rats (open bars). Values are mean ± SD.
Fatty (OLETF) strain. *Diabetes, 41:* 1422-1428.