Ultrastructural studies on lysosomes in retinal Müller cells of streptozotocin-diabetic rats

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Four Wistar inbred rats made diabetic by an injection of streptozotocin and four control rats were killed after an experimental period of 12 months. Eye tissues were prepared for examination and detection of acid phosphatase (AcPase) by electron microscopy. In the retina of control animals, the Müller cell cytoplasm had a small number of highly electron-dense bodies. AcPase reaction products were seen on these dense bodies and on Golgi lamellae of Müller cells. In the diabetic rat retina, a greater number of these lysosome-like bodies were seen, especially in cell processes adjacent to capillaries and in those at the vitreoretinal interface. Increased deposits of AcPase reaction products were detected on Golgi lamellae, smooth endoplasmic reticulum, and highly electron-dense bodies. The functional significance of a marked increase in lysosomal enzymes within Müller cells is uncertain. The phenomenon may occur in order to eliminate cellular debris derived from necrotic pericytes and to digest excessive glycogen accumulated in the retina under diabetic conditions.

Key words: experimental diabetes, streptozotocin, Wistar inbred rat, diabetic retinopathy, ultrastructure; Müller cells, lysosomes, acid phosphatase

Müller cells have been likened to glial cells for the role they play as interstitial supporting elements for retina neurons.1-4 Müller cells contain more ribosomes and endoplasmic reticulum than do any other retinal elements except pigment epithelium,1-4 making them capable of increased protein synthesis. They also synthesize and store glycogen in order to furnish glucose to retinal neurons.5-6

In the diabetic retina, Müller cells are infiltrated by basement membrane–like material.7-10 Intracytoplasmic deposits of highly electron-dense bodies (resembling lysosomes) have been found near capillary lesions in the retina of diabetic humans.11 Lysosomes associated with retinal metabolic disorders have been well studied.12-18 The aim of this study is to elucidate the characteristics of the lysosome-like bodies in retinal Müller cells of diabetic rats.

Materials and methods

Four 6-week-old male Wistar inbred rats (100 to 120 gm), obtained from Charles River Breeding Laboratories, Wilmington, Mass., were injected with streptozotocin (50 mg/kg) (Sigma Chemical Co., St. Louis, Mo.) as described previously.19

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Four age- and sex-matched littermates served as controls.

At 12 months, the rats were killed by cardiac perfusion with 25% Karnovsky's fixative. Immediately thereafter, both eyes were enucleated and immersed in 50% Karnovsky's solution for 2 hr. During fixation, the globes were opened and cut into small specimens for electron microscopy. After fixation, small blocks were rinsed overnight in 0.1M phosphate buffer (pH 7.4) with 5% sucrose, postfixed with 1% osmium tetroxide, and embedded in Epon 812. Sections were double-stained with uranyl acetate and lead citrate.

Fellow eyes were used for histochemical demonstration of acid phosphatase (AcPase). After fixation, they were transferred into 0.05M cacodylate buffer (pH 7.4) containing 5% sucrose and rinsed overnight. The blocks were cut into 40 μm sections by a cryostat at —20° C. Tissues were then incubated at room temperature for 60 min in modified Gomori medium20 with β-glycerophosphate as a substrate. Control tissues were incubated in medium without β-glycerophosphate or in medium supplemented with 0.01M sodium fluoride. (The tissues were rinsed in 0.1 M cacodylate buffer containing 5% sucrose, postfixed for 30 min in 1% osmium tetroxide, dehydrated, and embedded in Epon. Sections were either unstained or stained with uranyl acetate.

Results

All streptozotocin-injected rats were hyperglycemic (>250 mg/100 ml, by Dextrostix) and glycosuric (>4+, by Tes-Tape) throughout the experimental period; control rats were not.

Control rats. Müller cell cytoplasm was rich in endoplasmic reticula and ribosomes and had highly electron-dense bodies with a single-unit membrane containing electron-dense, fine, granular material (Fig. 1, A, arrows). AcPase was detectable on these dense bodies and on Golgi lamellae (Fig. 1, B, short arrows) of Müller cells and their extended processes (Fig. 1, C, arrows).

Diabetic rats. The highly electron-dense bodies of Müller cells were identical ultrastructurally to those in control rats. They were more numerous in cell processes extending to the outer and inner limiting membranes and were accompanied by an increased number of glycogen granules (Fig. 2, A). The dense bodies accumulated in the cell processes surrounding retinal vessels, especially the capillaries (Fig. 2, A). Often they appeared to be connected to each other (Fig. 2, A, long arrows) and to be continuous with the smooth-surfaced endoplasmic reticulum (Fig. 2, A, short arrow). Some contained vesicular or membranous structures (Fig. 2, B, long arrow); most, however, were filled with electron-dense, granular material (Fig. 2, B, short arrows). Dense bodies were generally small (about 1000 to 2000 Å in diameter), although their diameters ranged from 500 to 4000 Å (Fig. 2, A and B). AcPase was deposited on the ovoidal structures (Fig. 3, A, long arrows) and Golgi apparatus (Fig. 3, A, short arrows) in the Müller cell. Reaction products were also prevalent in the Müller cell processes facing the vitreous (Fig. 3, B).

Discussion

Müller cells in the normal retina have been characterized by radial spanning cytoplasm containing fibrils 100 Å in diameter, well-developed smooth endoplasmic reticulum, and glycogen granules.1-4 Müller cells have been considered a primary source of glycogen for retinal neurons.5, 6 The present study demonstrated that Müller cells contain electron-dense bodies that closely resemble lysosomes. AcPase was detectable on these dense bodies and on Golgi apparatus. These findings indicate that Müller cells produce more lysosomal enzymes under diabetic conditions. Müller cells per se may have the ability to digest, and thereby eliminate, residual bodies or toxic substances, whereas pigment epithelial cells constitute a phagosomal system in the outermost retina.11-13 Vascular cells, especially pericytes, undergo necrosis in diabetic retinopathy.20 The so-called secondary lysosomes may increase in number in the pericapillary Müller cell processes, although this was not the case in our study. The lysosomes seen were primary lysosomes which were rather small, ranging from 1000 to 2000 Å in diameter. Lysosomal enzymes may increase not only to digest cellular debris derived from necrotic vascular...
Fig. 1. Micrographs of control tissue. A, Highly electron-dense bodies (arrows) with a single-unit membrane containing fine granular material in a Müller cell (MC). Note electron-dense cytoplasm and angular nucleus of MC. N, Nerve cell. B, AcPase on Golgi lamellae (short arrows) and ovoidal structures (long arrows) of a Müller cell (MC) in the inner nuclear layer. C, Several AcPase reaction–positive ovoidal and tubular structures (arrows) in the Müller cell (MC) process facing the vitreous cavity (V).
Fig. 2. Micrographs of diabetic tissue. A, Increased number of highly electron-dense bodies (1000 to 2000 Å in diameter) in Müller cell (MC) processes around a capillary. Some are connected to each other (long arrows) and are continuous with tubular structure (short arrow). Note numerous glycogen granules in MC cytoplasm. L, Lumen; E, endothelial cell; P, pericyte. B, Highly electron-dense bodies containing fine granular material (short arrows) in Müller cell (MC) process. A dense body (measuring about 3600 Å) (long arrow) contains membranous, granular structures.
Fig. 3. Micrographs of diabetic tissue. A, Increased amount of AcPase reaction products on Golgi lamellae (short arrows) and ovoidal structures (long arrows) of Müller cell (MC) in the inner nuclear layer. B, Numerous AcPase-positive ovoidal and tubular structures (arrows) of Müller cell (MC) process facing vitreous cavity (V).
cells but also to break down the excessive glycogen that accumulates in Müller cells under diabetic conditions. The increase in lysosomes around capillaries can be explained by the idea that the capillary endothelium is the primary site of breakdown of the blood-retinal barrier in diabetic retinopathy. The vitreous has been reported to contain no lysosomal enzymes under normal conditions. Knowledge of pathophysiological alterations of the vitreous is still limited despite the importance of the vitreoretinal relationship in various eye diseases.

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