Fine Structure of the Retino-Optic Nerve Junction in Dogs

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MATERIALS AND METHODS

Fourteen adult dogs used in the present study were crossbred and medium-sized. Animals were anesthetized with sodium pentobarbital (Nembutal) and perfused with 2% paraformaldehyde-2.5% glutaraldehyde mixture in a 0.1 M phosphate buffer (pH 7.4) through the common cervical artery. The eyeball with the optic nerve was removed. The anterior half of the eyeball and vitreous body were cut away, and the posterior half and optic nerve were placed into the above fresh fixative at 4°C overnight or longer. The tissue was placed in buffered 1% osmium tetroxide (2–3 hr), rapidly dehydrated in a graded series of ethyl alcohols, and mildly dehydrated in a graded series of ethyl alcohols, and then the central part of the lamina cribrosa, or the scleral canal in animals without a distinct lamina cribrosa [9, 10]. In mammals, the optic nerve fibers in ION and the retina are generally thinner than those in EON, and oligodendroblasts cannot migrate into ION in embryo.

Well known exceptions are the myelinated fibers distributed in the retina and ION in rabbits [3, 4], and the myelinated fibers contained in ION in dogs [8, 17]. In comparison with the rabbit optic nerve and retina, there are few morphological studies of the optic nerve head and the nerve fiber layer in the retina of dogs, and no electron microscopic observations. The aim of this study was to describe a fine structure of the retinal nerve fiber layer and the optic nerve in dogs with special interest to myelination in the optic nerve fibers.

RESULTS

Extraocular Optic Nerve (EON): Astrocytes showed a typical fibrous type (Fig. 1). The nuclei of astrocytes were light, bean-shaped, and the nuclear envelope was often folded. The cytoplasm was relatively wide with broad cytoplasmic processes arising from the perikaryon. The most prominent cytoplasmic component was the numerous glial filaments that occurred throughout the perikaryon and extended as parallel arrays into the processes. The usual organelles were found in the perikaryon including the mitochondria, Golgi complex, and rough endoplasmic reticulum. Few microtubules were observed.

Oligodendrocytes were of the medium type according to Mori and Leblond [13] (Fig. 2). Oligodendrocytes were moderately dense. That density was caused by a large number of fine granules occupying the space between other organelles. The nuclei were round or oval, and the nuclear chromatin tended to clump. In contrast to astrocytes, oligodendrocytes had a mostly smooth contour with a few pro-
cesses. The cytoplasm was relatively abundant with many organelles. The obvious cytoplasmic components were well developed rough endoplasmic reticulum, free polysomes, Golgi complex, and mitochondria. Microtubules were conspicuous, unlike in the surrounding astrocytes.

The optic nerve fibers were largely myelinated and unmyelinated fibers were markedly thinner than myelinated ones.

**Intraocular Optic Nerve (ION):** For convenience of a description, ION was divided into retinal, choroidal, and scleral sections based on the respective adjoining components (Fig. 3). In the scleral section, the optic nerve fiber bundles located within the pores of the lamina cribrosa.

In most mammals, the peripheral optic nerve is bottle-shaped, with the neck corresponding to ION and the body to EON. However, in the dog, ION was only slightly thinner than EON (Fig. 3).

The scleral and choroidal sections consisted largely of myelinated fibers but comprised more unmyelinated fibers than in EON. In the retinal section myelinated fibers decreased markedly in number and completely disappeared from the narrow part along the retina. Myelinated and unmyelinated fibers mingled together in the retinal section and axons of both types had frequently similar in diameter. As for myelinated fibers, thickness of myelin sheath of axons with a similar diameter was various (Fig. 4).

In the scleral ION astrocytes were similar to those in EON. In the choroidal section, astrocytes were of a typical fibrous type and richer in cytoplasm than astrocytes in EON. Their processes were noteworthy in number and longer than those in EON. The perikaryon contained relatively many Golgi complex, mitochondria, rough endoplasmic reticulum polysomes, microtubules and intricate bundles of gliofila-
ments (Fig. 5). Astrocytes contained often many microtubules (Fig. 5). In the retinal section, the astrocytes were characterized by a smaller amount of cytoplasm with sparser gliofilaments than in the scleral and choroidal sections, and similar to the retina. However, they were regarded as fibrous type because their processes tended to be slender and rectilinear. Oligodendrocytes in the scleral and choroidal sections were similar to those in EON belonging to the medium type classified by Mori and Leblond [13] (Fig. 6). In the retinal section oligodendrocytes decreased in number in correlation with the decrease in myelinated fibers. Moreover, oligodendrocytes close to the retina changed from the medium to light type. The light type of oligodendrocytes often contained few microtubules (Fig. 7). There were a few light oligodendrocytes that were difficult to distinguish from astrocytes in the same area because astrocytes contain only a few gliofilaments.

Nerve Fiber Layer in the Retina: There were no myelinated fibers in the nerve fiber layer. Unmyelinated fibers tended to be thicker than that in EON.

The distal processes of Muller cells were vertically oriented, bundling the ganglion axons and abutted against the vitreous body by the end feet. The distal processes were very electron-dense and characterized by filaments and smooth endoplasmic reticulum.

The cell bodies of astrocytes were almost all located in the ganglion cell layer, between the fiber bundles in the nerve fiber layer, or close to the vessel. The nuclei of the astrocytes were light and exhibited a fine-granular homogeneously distributed chromatin. The perikarya were relatively narrow and rather pale, as was the axoplasm of the ganglion axons including a small Golgi complex, mitochondria, and short cistern of the rough endoplasmic reticulum (Fig. 8). The astrocytic processes were fewer than in EON and were not involved in the partitioning of nerve fibers into fascicles.
The fasciculation of fibers was done by Muller cell processes. The astrocytic processes were easily distinguished from the Muller cell processes by electron density. Glial filaments were sparse in astrocytes.

**DISCUSSION**

In the submammals except for amphibians, myelinated fibers are predominant in EON, decrease in number in ION, and are a few in the optic nerve fiber layer of the retina [2, 8, 14, 20]. In the chicken, myelinated fibers make up 12.5% of the nerve fibers of the optic nerve fiber layer [11]. But it should be emphasized that ION and the retinal optic nerve fiber layer in submammals contain constantly myelinated fibers in some extent. In almost all mammals, however, the optic nerve fibers are largely myelinated in EON as in submammals, but unmyelinated in ION and the retina. Myelination in the retinal nerve fiber layer is found in spontaneous [1, 19, 21] and experimental [15, 16] cases in mammals. In the spontaneous cases, a small number of axons in the retina become myelinated by Schwann cells which have gained access to the retina. This aberrant myelination in the mammalian retina shows that all optic nerve fibers are unmyelinated for lack of oligodendrocytes. It has been suggested that oligodendroblasts are prevented from migrating from EON into ION so that normal myelination does not occur [6, 18]. The hypothetical barrier preventing migration of oligodendroblasts in embryo is peculiar to mammals.

In this study, it was confirmed by electron microscopy that myelinated fibers were clearly dominant in the scleral and choroidal sections of ION in dogs. Myelinated fibers decreased suddenly in the retinal section of ION and were

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**Fig. 7.** Oligodendrocyte in the retinal section of the intraocular optic nerve is of a light type that is characterized by pale cytoplasm and nucleus, and irregular contour of cytoplasm. Scale bar=1 μm.

**Fig. 8.** Astrocyte in the optic nerve fiber layer in the retina. Scale bar=1 μm.
absent from a narrow area adjoining the retina. Thus, the barrier to migration of oligodendroblasts into the retina, if any, is located in the periphery of the retinal section of ION. While the precise factors that influence migration of oligodendroblasts into ION are unknown, some assumptions have been proposed. The transition zone from myelinated to unmyelinated fibers in the optic nerve is characterized by a dense meshwork of fibrous astrocyte processes and a defect in the blood optic nerve barrier [5, 7, 9, 12]. The lamina cribrosa has been suspected of acting as the barrier preventing migration of oligodendroblasts into the retina during development because the lamina is located in the transition zone from the myelinated to unmyelinated part of the optic nerve in many mammals including humans [16]. Furthermore, the subpopulation of astrocytes in the lamina has been suspected of having a barrier function to myelination during optic nerve maturation [6, 12, 22]. Since in the dog optic nerve the lamina cribrosa is obvious [8] and oligodendrocytes distribute in ION, the lamina is not a candidate for the migration barrier. The peripheral area of the retinal section along the retina, which is a putative site for the migration barrier in the dog, had sparse astrocytes as well as their processes. Thus, our results argue against the astrocyte theory for the migration barrier.

The blood optic nerve barrier is incomplete in the optic nerve head because the connective tissue of the lamina cribrosa and pial septa lies between the capillaries and the perivascular glial limiting membrane [7]. Plasma protein derived from the defect in the blood optic nerve barrier may be responsible for the barrier to migration of oligodendroblasts [6, 9, 10, 16]. Since there is no connective tissue in the choroidal and retinal sections of ION in the dog, the blood optic nerve barrier in this region is probably normal. We have concluded that the plasma protein is not implicated in the barrier formation.

If the retinal nerve fiber layer consisted predominantly of myelinated fibers, the retina would be opaque and vision disorders would occur. In submammals the retina contains oligodendrocytes and myelinated fibers but is not opaque. To maintain transparency of the retina, the majority of retinal ganglion axons have to remain thinner than the critical size that induces myelination. In mammals as well as submammals, the optic nerve fibers within the eyeball are thinner than those in EON [17]. For the maintenance of mammalian retinal transparency, it has been stressed that oligodendrocytes do not distribute in the retina. However, it may be crucially important that the growth in thickness of the optic nerve fibers is suppressed in the retina through all vertebrates. In mammals with an unmyelinated ION, the growth of the nerve fibers is suppressed at more peripheral part than the location of the barrier for migration of oligodendroblasts. That is to say, in these mammals the barrier looks like to suppress both of the migration of oligodendrocytes and the growth of the thickness of the nerve fibers. In the dog, the barrier to migration of oligodendroblasts could be located in the narrow area of the retinal section along the retina, while the growth of the optic nerve fibers was suppressed through the whole span of ION. Thus, the mechanism by which the optic nerve fibers in ION remain thinner is obscure, but would be different from the barrier to migration of oligodendroblasts.

REFERENCES


