Effects of chronic denervation on the histology of canine extraocular muscle

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The histologic and enzyme histochemical characteristics of chronically denervated dog extraocular muscle (EOM) were studied. Our results show atrophy of coarse and granular fibers, with relative sparing of fine fibers. Thus experimentally denervated EOM does not demonstrate the familiar changes of denervated limb muscle but shows prominent atrophy of two of the three EOM fiber types. (INVEST OPHTHALMOL VIS SCI 22:701-705, 1982.)

Key words: extraocular muscle, third-nerve denervation, dog, fiber atrophy

Interest in the histologic changes of extraocular muscle (EOM) produced by experimental denervation was stimulated by the controversy over the etiology of chronic progressive external ophthalmoplegia (CPEO). Failure to recognize the differences between normal EOM and normal limb muscle led to difficulty in the interpretation of biopsy material from the EOM of patients with CPEO. It is now clear that a separate experimental and clinical experience in the study of EOM must be acquired.

Previous histologic and enzyme histochemical studies of EOM after experimental denervation have failed to show fiber atrophy or type grouping. Review of these studies led us to suspect that reinnervation was not prevented for a long enough period to allow chronic changes to develop. The present study is designed to define the alterations in dog EOM produced by 3 months of complete denervation.

Materials and methods

Five full-grown mongrel dogs were subjected to an intracranial lesion of the left oculomotor nerve. They were anesthetized with intravenously administered thiopental sodium, a left temporal craniectomy was performed, and the temporal lobe was retracted medially. With the operating microscope, the left oculomotor nerve was identified as it entered the cavernous sinus and its course was followed back toward the midbrain. The pupillary reactions to light were checked, then the nerve was lifted off the posterior part of the sinus (in the dog, the sinus extends under the oculomotor nerve and behind the posterior clinoids) and cut as close as possible without disturbing the sinus itself. The proximal part of the nerve was followed back and a second cut was made close to the midbrain, thus completely removing a section of nerve about 5 mm in length. The proximal segment of nerve was positioned infratentorially. The lesion abolished the pupillary light response in all animals. Hemostasis was achieved and the wound was closed in layers.

Ocular motility and pupils were examined regularly over the next 3 months. On the first postoperative day there was complete left ptosis, no pupillary light response, and the eye was deviated inferiorly and laterally in all animals. Over the next 2 weeks, the ptosis became less marked in all animals, but the left pupil remained unresponsive and the left eye remained positioned down and out. One dog died of an unrelated cause and did not undergo autopsy. The four remaining dogs de-
veloped the habit of fixing and following with their right eye and turned their heads to use this eye preferentially. When these dogs looked to the right, intorsion and some movement down and in from the resting inferolateral position was noted from 1 month until the time of biopsy. No ability to elevate, adduct (beyond the mentioned above), or extort the left eye was observed. The left pupil remained unresponsive to direct and consensual light stimulation.

Three months after the intracranial procedure, all dogs were anesthetized with thiopental sodium and biopsies of the right and left inferior oblique muscles were performed. This muscle was chosen for biopsy because of its accessibility, short tendinous insertion, and its use in human subjects. A lateral canthotomy was performed, the lateral conjunctiva was incised at the limbus, and Tenon's capsule was entered. A partial peritomy was performed and the lateral rectus was hooked. The two-headed insertion of the inferior oblique was identified, and a full-thickness 1 cm biopsy was taken between the insertion and point of nerve entry. The two muscle ends were released and the canthotomy was closed. Two weeks later the animals were sacrificed and examined in autopsy.

Normal and denervated muscle from each dog was quick frozen in isopentane and immersed in liquid nitrogen. Serial 10 μm cryostat sections were stained with hemotoxylin and eosin, modified Gomori trichrome, oil red-O, periodic acid-Shiff, adenosine triphosphatase (ATPase), pH 9.4, and nicotinamide adenine dehydrogenase-tetrazolium reductase (NADH-TR).

In each muscle, a representative area of central fascicles containing a mixture of fiber types was selected for microscopic study. Fifty fibers of each type were identified by the modified trichrome, NADH-TR, and ATPase reactions of fiber typing. The maximum, lesser diameter of 50 fibers of each type was determined for each biopsy, and histograms were constructed comparing the normal and denervated sides (Fig. 1).

Results

Our findings in all biopsies of normal dog EOM (Figs. 1 and 2) recapitulate those of previous investigators of normal primate and human EOM and will only be summarized. EOM fibers were readily typed by enzyme histochemical criteria into three groups: coarse, granular, and fine. Coarse fibers averaged 23 μm in diameter and were most plentiful at the periphery. Granular fibers were the largest (average 32 μm) and the most plentiful in central fascicles. Fine fibers were seen almost exclusively in the central fascicles and averaged 23 μm in diameter.

Denervated EOM showed striking atrophy of the coarse and granular fibers, many of which had an angular configuration (Fig. 2). The reduction in mean diameter of these fibers compared with the normal side was statistically significant (p < 0.001 by the two-tailed student's t test) in all four dogs (Fig. 1). Fine fibers were relatively spared from the atrophic process, with no statistically significant reduction in mean fiber diameter in three of four dogs (Fig. 1). Hypertrophied fibers, type grouping, and group atrophy were not found. Increased perimysial and endomysial connective tissues were prominent in denervated EOM, and mild lymphocytic infiltrates were identified in most specimens. There were no necrotic fibers. Pyknotic nuclear clumps and target fibers were not seen.

Autopsy revealed that the sectioned ends of the oculomotor nerves had not established continuity, and EOMs innervated by the sectioned oculomotor nerves were darker, thinner, and softer than normal muscle.

Discussion

In recent years, histologic and enzyme histochemical studies of skeletal muscle have led to acceptance of a basic two fiber-type system with subgroups. Changes in the pattern of distribution, relative frequency, and size of these fiber types and subtypes have been described for a number of neuromuscular disorders.

EOM is classified by fiber types according to the same techniques. The system used here is that devised by Durston and used by Ringel et al. in his series of experiments, wherein three fiber types are described. Details of these three groups of fibers in the monkey and for man have been described. Although no exact correlation exists between limb and EOM fibers, fine and granular fibers most closely correspond to types 1 and 2 limb fibers, respectively. Coarse fibers have no histochemical counterpart in limb muscle and are further unique in having multiple nerve endings.
Fig. 1. Fiber diameter histograms of denervated (o) and control (•) inferior oblique muscles for coarse, granular, and fine fiber types. Mean fiber diameter of denervated (M_D) and control (M_C) muscles are statistically different at (p < 0.001) level for coarse and granular fibers in all dogs. Mean fiber diameters of fine fibers were significantly different in only one of four dogs.

EOM biopsy has been used in the clinical study of CPEO. The history of this nosologic entity illustrates the difficulties encountered when limb muscle criteria are applied to EOM biopsy material. CPEO was, at the onset, described as a neural disorder. Biopsies of levator and EOM swung the opinion toward classification as myopathy by 1950. Cases of CPEO with clear neural involvement led to experiments to establish the validity of the criteria for calling EOM neuropathic or myopathic.

EOM pathologic studies on the myotonic mouse have been carried out and fiber splitting, central nuclei, and hypertrophic fibers have been identified. Drachman et al. studied the effect of crushing or avulsing one oculomotor nerve in the dog. EOM was taken at time intervals of 2 weeks, and 1, 2, 3, 6, and 12 months after the procedure. They found no type grouping, group atrophy, or decrease in fiber size, although inflammatory infiltrate, central nuclei, and other myopathic changes were observed. The most prominent
Fig. 2. For legend see facing page.
changes occurred early in the study, and by 3 months some return of oculomotor nerve function was noted. It is possible that the findings revealed the early stages of denervation in EOM and the long-term effects were obscured by substantial reinnervation. The study made clear the need for experimentally established criteria for assessment of EOM biopsy.

Ringel et al. studied the histochemistry and acetylcholine receptor distribution in normal and denervated monkey EOM. Study intervals were 2, 6, and 13 weeks after severing one oculomotor nerve. At no time was a difference in fiber diameter between normal and denervated EOM noted. Type grouping or group atrophy was not observed. The most prominent changes were increased numbers of vesicular nuclei, prominent inflammatory cell infiltrates, and coarsening of intermyofibrillar architecture. Conclusions about long-term denervation in the study are difficult because of evidence of aberrant reinnervation in the animal sacrificed at 13 weeks.

Ringel's study of humans with CPEO showed consistent decrease in fiber size in biopsy specimens. Of eight patients with CPEO, five had decrease in fiber size of all types, one had small fine and granular fibers and normal coarse fibers, one had small coarse and granular fibers with normal fine fibers, and one had no fiber shrinkage.

Our surgical technique was designed to prevent regrowth of the severed oculomotor nerve for a prolonged period. None of our animals showed return of function, and at autopsy, none of the nerves had reestablished continuity. In denervated EOM, granular and coarse fibers underwent marked atrophy with increased numbers of angular fibers. Fine fibers were spared from the atrophic process and were the largest fibers present. The reason for fine fiber sparing is not apparent, but one possible explanation is that these fibers may be innervated by nonmedullated nerves, which have been shown to survive third-nerve section for at least 21 days. It has been suggested that these fibers are from the sympathetic system and that they innervate muscle fibers. Specific studies will be required to investigate this explanation.

REFERENCES


Fig. 2. A, Normal dog EOM, central fascicle. Coarse and granular fibers are dark, fine fibers are light. Fine fibers have the smallest average size on this normal specimen. Stipled appearance is due to ice crystal artifact. (ATPase at pH 9.4; ×344.) B, Third-nerve denervated dog EOM, central fascicle. Fine fibers are normal size, but coarse and granular fibers show marked atrophy. Stipled appearance is due to ice crystal artifact. (ATPase at pH 9.4; ×344.)