Posterior Attachment of Ciliary Muscle in Young, Accommodating Old, Presbyopic Monkeys

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The authors studied the posterior attachment of the ciliary muscle in seven young (3–10 yr) and five old (26–34 yr) rhesus monkeys by light microscopy and electron microscopy. Posterior attachment of the muscle bundles consisted of elastic tendons, exclusively. The elastic tendons were continuous with the elastic lamina of Bruch’s membrane and were also connected by smaller elastic fibers to an elastic meshwork that surrounds the pars plana vessels. In some areas, the tendons formed focal contacts with the endothelial cells. The authors found that in old eyes, the tendons and the elastic fibers of the posterior ciliary body showed pronounced structural changes. The tendons appeared thickened, showed increased amounts of associated microfibrils, and were surrounded by dense layers of thick collagen fibrils. An increased amount of collagen fibrils was also seen between the elastic layer of Bruch’s membrane and the pigmented epithelium. A mechanical link between those collagen fibrils and the elastic fibers is suggested by the presence of osmiophilic points of contact. The age-related increase in elastic fibrillar material could cause decreased compliance of the posterior insertion of ciliary muscle and could be an essential factor for presbyopia in rhesus monkeys. Invest Ophthalmol Vis Sci 32:1678–1692, 1991

The function of the primate ciliary muscle in accommodation depends on the inward movement of the anterior muscle during contraction.1 The movement of the muscle requires a remodeling of its inner structure. Morphometric studies on meridional sections of human2 and monkey eyes,3,4 after treatment with pilocarpine and atropine show that contraction of the ciliary muscle is associated with an increase in area of the circular and reticular portion and a decrease in area of the meridional portion. Additionally, the meridional portion shortens and the posterior end of the ciliary muscle moves anteriorly. To support the anterior movement of the muscle, a firm anterior attachment is necessary. This is supplied by broad inelastic collagenous tendons, which insert within the scleral spur, and via the trabecular meshwork, into the peripheral cornea.5,6,7,8 In contrast, the posterior attachment consists of elastic tendons.2,9,10,11,12 Due to their extensibility, the contracting muscle can move forward during accommodation. The stretched posterior elastic tendons pull the ciliary muscle backwards during relaxation and desaccommodation.

We have shown that with increasing age, the ciliary muscle of the rhesus monkey (Macaca mulata) loses its anterior inward movement in response to pilocarpine.4 Video recording of ciliary body coronal width, after central electric stimulation of young (3-yr-old) and old (>20 yr) iridectomized rhesus monkeys, shows loss of measurable ciliary body movement with age, indicating loss of effective ciliary muscle contraction.13 The age-related decline in structural response parallels the loss of functional accommodative response to pilocarpine or carbachol.14 Thus, loss of ciliary muscle movement seems to be involved in the loss of accommodation and the development of presbyopia in this species.

Age-related changes in the ultrastructure of the ciliary muscle cells and intramuscular connective tissue of the rhesus monkey are moderate and do not account for the loss of structural and functional responses.15 An increased inelastic posterior fixation, however, might restrict ciliary muscle anterior inward movement. We, therefore, investigated the ultrastructure of the posterior insertion of the rhesus monkey both in young (3–10 yr) and in old (26–34 yr) animals.
Material and Methods

The eyes of seven young adult (3-10 yr) and five old (26, 26, 30, 31, 34 yr) rhesus monkeys of both sexes were studied. The monkeys were from caged colonies of the Wisconsin Regional Primate Research Center (Madison, WI). The young animals were killed in conjunction with various nonocular protocols, whereas the elderly animals were debilitated and were euthanized. Anesthesia for in vivo enucleation and systemic perfusion was intramuscular ketamine HCl 15 mg/kg and intravenous pentobarbital Na 25 mg/kg. The animals were killed by a pentobarbital overdose. These experiments were conducted in accordance with the ARVO Resolution on the Use of Animals in Research.

Three of the animals (8, 10, 34 yr) were perfusion fixed via the heart with Ito's fixative, after perfusion with heparinized NaCl. The eyes of the remaining animals were fixed by immersion immediately after in vivo enucleation. Windows were cut in the posterior sclera and the cornea of these eyes, after which the entire eyes were placed in Ito's fixative for at least 3 hr before dissection, to preserve the architecture of the ciliary muscle and its posterior attachment to Bruch's membrane.

The eyes were sent to us by Professor P. L. Kaufman (Department of Ophthalmology, University of Wisconsin, Medical School, Madison). No ocular abnormalities, other than senile cataract and peripheral cystoid retinal degeneration, were seen clinically or histopathologically in the old animals. The old monkeys received, in vivo, one drop of commercial ophthalmic 10% pilocarpine hydrochloride topically in one eye and 1% atropine sulfate in the other eye, 45 min and 30 min, respectively, before fixation began. In each case, the pupils responded appropriately. In the eyes of two of the old animals (26, 34 yr), we had shown a marked decrease in or no morphologic response to pilocarpine.

To ensure that the ciliary muscle remained anchored anteriorly and via Bruch's membrane posteriorly, four sectors running from the posterior to the anterior pole, with a width of approximately 5 mm at the equator, were cut from each quadrant of the eyes. These experiments were conducted in accordance with the ARVO Resolution on the Use of Animals in Research.

From both paraffin and Epon specimens, sections for light microscopy were cut with different planes of orientation of the sections. Meridional, frontal (A), oblique-frontal (B), and oblique-tangential (C) sections were cut.

Fig. 1. Schematic drawing of the rhesus monkey ciliary body to show the planes of orientation of the sections. Meridional, frontal (A), oblique-frontal (B), and oblique-tangential (C) sections were cut.
Fig. 3. Oblique-tangential section of the posterior attachment of the ciliary muscle in a young monkey. The elastic tendons (arrows) that originate from the muscle bundles (CM) are connected by smaller elastic fibrils to a network of elastic fibers (asterisk) surrounding the vessels of the posterior ciliary body. This network is continuous with the elastic layer of Bruch's membrane next to the pigmented epithelium (PE). Due to the obliquity of cut, the elastic lamina is partly resolved into its substructure, which consists of a meshwork of elastic fibers. In some areas, elastic tendons are in close contact (arrowheads) with the walls of the pars plana capillaries (C) (paraffin section, resorcin-fuchsin/van Giesson stain, x1000).

Fig. 4. Posterior attachment of the meridional ciliary muscle portion in rhesus monkey eyes. The meridional muscle bundles (CM) form elastic tendons (arrows) that are continuous with the elastic layer of Bruch's membrane. Tendons of different bundles are connected by smaller elastic fibers to an elastic network that surrounds the vessels of the pars plana. Thick elastic tendons are in close contact with the endothelium of the vessels.
orientation, eg, meridional, frontal, oblique-frontal, and oblique-tangential (Fig. 1). Generally, the paraffin sections were 5 μm thick, and the Epon specimens were 1 μm thick. In each plane, serial sections were cut through the whole muscle and the surrounding connective tissue, both in paraffin and in Epon specimens. The paraffin sections were stained with a combined Weigert’s resorcin–fuchsin/van Gieson stain for elastic fibers and smooth muscle cells. Other standard elastic stains used were Gomori’s aldehyde fuchsin and Verhoeff’s iron hæmatoxylin. The Epon sections were stained with Richardson’s stain and with modified Weigert’s resorcin–fuchsin and Verhoeff’s iron hæmatoxylin.

Additionally, ultrathin sections were cut of the ciliary muscle and its attachment to the surrounding connective tissue. This allowed for a detailed examination of the muscle fibers and their connection to the tendon. The images show the muscle cells with their long cytoplasmic processes and invaginations. The area of magnification is shown (circle) in Figure 5B (electronmicrograph, ×3300).

Fig. 5A. Oblique-tangential section of an elastic tendon (ET) of a ciliary muscle bundle (CM) form the meridional portion in a young monkey. In the region of the myotendinous junction, the muscle cells show tapered ends with long cytoplasmic processes and invaginations (arrows). The area of magnification is shown (circle) in Figure 5B (electronmicrograph, ×3300).
connective tissue, using the planes of sectioning described above. The sections were treated with lead citrate and uranyl acetate. For electron microscopic investigation, JEOL (JEM 100 b) and Zeiss (EM 902, Germany) electron microscopes were used.

Results

Young, Adult Animals

Light microscopy: In the eyes of rhesus monkeys, the meridional portion of the ciliary muscle ends posteriorly about 0.5–0.9 mm anterior to the ora serrata. From the ends of the posterior-most muscle bundles, tendons originate that stain positive with all three elastic fiber stains used. The band-like elastic tendons have a smallest diameter of 0.6–0.8 μm, which is seen in meridional sections, and a widest diameter of 2–2.5 μm. The tendons are continuous with the elastic lamina of Bruch’s membrane (Fig. 2). The elastic tendons of different bundles are connected by smaller elastic fibers (0.5–0.7 μm) to an elastic network, seen in the stroma between ciliary muscle and pigmented epithelium. The elastic network surrounds the efferent vessels of the ciliary body in the pars plana and is connected with the elastic network of Bruch’s membrane (Figs. 3, 4). In some areas, the elastic tendons are in close contact with the pars plana capillaries (Figs. 3, 4). In the posterior part of the reticular portion, the muscle bundles also form elastic tendons, which are connected with an elastic network around the ciliary body capillaries, and insert in the elastic lamina of Bruch’s membrane. The elastic tendons are, however, shorter than those of the meridional portion.

Electron microscopy

Meridional Portion

In the region of the myotendinous junction, the individual ciliary muscle cells show tapered ends with long cytoplasmic processes and long furrows and invaginations (Figs. 5A, B). The elastic tendons originate partly from the invaginations of the muscle cells and partly from the outer surface of the tips (Fig. 5A). Thus, the tips of the cells are completely embedded in elastic material. In regions, where the elastic tendons are connected to the muscle cells, the cell membrane forms dense bands with adhering myofilaments. In areas where the cell membrane is specialized by dense bands, no intervening basal lamina is seen between the elastic tendons and the cell membrane (Fig. 5B).

The elastic tendons show the typical ultrastructural characteristic for elastic fibers. They consist of a central, amorphous, moderate osmiophilic core and a coat of microfibrils (Fig. 5).

This is the same for the fibers of the elastic network, which surround the capillaries of the ciliary body and are connected with the elastic tendons. The fibers of the elastic network are also connected with the basal lamina of the endothelial cells by small microfibrils. Occasionally, the thick elastic tendons run close to the basal lamina of the endothelial cells of the pars plana capillaries (Fig. 6A). In some areas, the elastic tendons contact the endothelial cell without intervening basal lamina. In these areas, the cytoplasm close to the cell membrane of the endothelial cell is more electron-dense than elsewhere (Fig. 6B).

A small amount of loosely arranged collagen fibers embedded in an electron-lucent ground substance ap-
Fig. 6A. Oblique-tangential section of the elastic tendons (ET) of the meridional ciliary muscle portion in a young monkey. The elastic tendons are in broad contact with the basal lamina of the endothelial cells of the pars plana capillaries (arrows). The area of magnification (circle) is shown in Figure 6B (electronmicrograph, ×3300).

Fig. 6B. Higher magnification of Figure 6A. The elastic tendon forms close points of contact with the endothelial cells. The elastic tendon appears to contact the cell membrane directly, and the cytoplasm close to the point of contact is more electron-dense than elsewhere (arrow, ×60,000).

appears among the ends of the muscle bundles, the elastic tendons, the capillaries, and the elastic lamina of Bruch's membrane (Fig. 7). The collagen fibrils have a diameter of 40–50 nm. Near the muscle cells, the collagen fibers show no apparent association with the elastic tendons and pass randomly among the meshes of the elastic network of the posterior ciliary body. Closer to Bruch's membrane, the collagen fibers are occasionally in contact with small bundles of microfibrils that emerge from protrusions of the elastic fibers. Between the elastic layer of Bruch's membrane and the pigmented epithelium, microfibrils and small col-
lagen fibrils can be seen (Fig. 8). The microfibrils form a fine filamentous connection between the elastic layer and the basal lamina of the pigmented epithelium (Fig. 8). Where the microfibrils are in contact with the elastic layer, the elastic fibers form extensions or protrusions (Fig. 8).

**Reticular Portion**

In the posterior part of the reticular portion, the elastic tendons are thinner, but they show the same ultrastructural characteristics as in the meridional portion. The myotendinous junction shows some differences to the meridional portion. The muscle cells form a single invagination or furrow at their tip, which is filled with elastic material. The adjacent cell membrane shows dense bands, whereas a basal lamina cannot be seen. The tendons are also seen adjacent to endothelial cells of the capillaries of the ciliary body and are connected to the basal lamina of the endothelial cells by microfibrils.

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**Fig. 7.** Oblique–tangential section of the posterior elastic tendons (ET) in a young monkey. The elastic tendons of different bundles are connected by smaller elastic fibers. Elastic tendons show an intimate association with the pars plana capillaries (arrows). A small amount of loosely arranged collagen fibrils (arrowheads) embedded in an electron-lucent ground substance is seen (electronmicrograph, x16,000).

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**Fig. 8.** Oblique–tangential section of Bruch’s membrane and the pigmented epithelium in the region of the attachment of the ciliary muscle tendons in a young monkey. Between the elastic layer (EL) of Bruch’s membrane and the pigmented epithelium (PE), bundles of microfibrils and several collagen fibrils are seen. The microfibrils emerge from protrusions of the elastic layer (arrows) and extend toward the basal lamina of the pigmented epithelium, forming a fine filamentous connection (electronmicrograph, x20,000).
Old Animals

Light Microscopy: In the three animals older than 30 yr, neither the elastic tendons of the meridional portion and the posterior reticular portion nor the elastic lamina of Bruch's membrane in the region of its connection with the tendons can be stained with the conventional histologic elastic fiber stains (Fig. 9). Also, when stained with Richardson's stain, the elastic fibers remain pale and do not stain intensely blue like the elastic fibers in young animals (Fig. 10). This is, however, only true case for the region of the posterior ciliary body. In contrast, the elastic lamina of Bruch's membrane posterior to the ora serrata and the elastic-like fibers of the trabecular meshwork show the same staining pattern as in young animals. The elastic tendons in the old eyes are markedly thickened, with a widest diameter of 4.5-5.5 μm. The tendons are not smooth as in young animals, but have a more irregular shape, with fuzzy borders and a notched appearance (Fig. 9). The elastic fibers of the elastic network in the posterior ciliary body are also thickened. The network does not show its normal architecture but appears disintegrated and fragmented (Fig. 9). The connective tissue between the elastic tendons of the reticular and meridional muscle bundles and the ciliary pigmented epithelium is markedly thickened and hyalinized (Figs. 9, 10). This finding was especially pronounced in the three animals older than 30 yr.

Electron Microscopy

Electron microscopic examination showed that all elastic fibers in the region of the posterior attachment of the ciliary muscle are changed when compared with elastic fibers in the same region in young animals (Figs. 11-15).

The homogenous component of the elastic fibers is electron lucent and is filled with fine fibrogranular material. Additionally, it contains scattered bundles of electron-opaque microfibrils, which coalesce to dark, electron-dense areas (Fig. 11). The microfibrils have a diameter of 10-12 nm and a periodicity of 15 nm. In contrast to young animals, bundles of microfibrils with similar diameter and periodicity are also seen at the attachment of the tendons to the muscle cells. The microfibrils connect the homogenous component of the elastic tendon with the muscle cells (Fig. 12A). Where dense bands are seen, they contact the cell membrane directly, without intervening basal lamina (Fig. 12B). Near the muscular insertion, the elastic tendons are embedded in a dense, granular osmiophilic ground substance that contains scattered collagen fibers (Fig. 12A). This is not seen in young animals. The amount of surrounding collagen increases posteriorly toward Bruch's membrane. In this region, the elastic tendons are almost surrounded by a coat of collagen fibrils (Fig. 13). The collagen fibrils have diameters of 200-250 nm (Fig. 13), are parallel to the elastic tendons, and pass through the meshes of the elastic network of the posterior ciliary body. Among the collagen fibrils, clusters of membrane-bound vesicles and granular and tubular structures can be seen (Fig. 13). These connective tissue changes are all characteristic for old animals, but are most pronounced in the three animals older than 30 yr.

Between the elastic layer of Bruch's membrane and the ciliary pigmented epithelium, there is a thick, dense collagen layer (Fig. 14A). The collagen fibrils show a mainly meridional orientation, running roughly parallel to the sclera. The collagen fibrils are attached to the elastic lamina anteriorly and to the
thickened basal lamina of the pigmented epithelium posteriorly. They are continuous with dense arrays of microfibrils that emerge from the elastic lamina of Bruch's membrane (Fig. 14B). The microfibrils have the same orientation as the collagen fibrils. In some areas, the collagen fibrils contact the elastic lamina without intervening microfibrils. The collagen fibrils appear to be anchored to the elastic layer by osmophilic material (Fig. 15). The alignment of the collagen fibrils suggests that they are under tension and exert a pull on the elastic lamina, allowing small protrusions. The collagen fibers extend toward the pigmented epithelium and show an intimate association with its thickened basal lamina.

In the eyes of all of the old animals studied, there was no difference in the ultrastructure of the posterior
Fig. 12A. Oblique-tangential section of the myotendinous junction of an elastic tendon in the region of the posterior attachment of the meridional portion of the ciliary muscle in a 34-year-old monkey. Muscle cell (C) and tendon are surrounded by electron-dense, amorphous, granular, extracellular material (asterisks) (electronmicrograph, ×7500).

Fig. 12B. Higher magnification of Figure 10A. At the myotendinous junction, bundles of microfilaments (asterisks) emerge from the homogenous component of the elastic tendon and extend deeply into the invaginations formed by the muscle cell (C). Where the microfilaments appear to be attached to the cell membrane, the basal lamina is interrupted (arrowheads) and dense bands with adhering myofilaments (arrows) are formed (×40,000).
attachment of the ciliary muscle. There was also no difference in the ultrastructure of this region between pilocarpine-treated and atropine-treated eyes.

Discussion

This study shows that the posterior tendons of the reticular and meridional muscle bundles of the ciliary muscle of the young rhesus monkey are purely elastic, with the typical histologic staining qualities and ultrastructural characteristics of elastic fibers found in other tissues. Thus, the tendons differ ultrastructurally from the anterior elastic-like tendons of the primate ciliary muscle, that are seen in addition to the anterior collagenous tendons.

Distinctive ultrastructural features of the posterior ciliary muscle tendons are the numerous projections and invaginations of the muscle cell membrane and the elongated membrane-bound dense bands in the myotendinous region. These features are also typical for the collagenous tendons of striated muscle cells. True elastic tendons of smooth muscle cells, however, have been described for the human arrector pili muscle and the homologous feather muscle of birds. In all of these elastic tendons, the muscle cells show similar ultrastructural features in the region of the myotendinous junction, but the mode of the elastic fiber attachment differs. In young animals, the ciliary muscle, the homogenous part of the elastic fiber, is in close contact with the cell membrane, and a basal lamina cannot be seen. In contrast, in the skin and feather muscle tendons, dense arrays of the elastin-associated microfibrils are embedded in the muscle cell basal lamina.

Elasticity of the posterior attachment of the ciliary muscle is important to allow forward movement of the muscle in accommodation and subsequent restoration of its length and position during desaccommodation. Desaccommodation implies that the tension is restored to the elastic components of the lens. Helmholtz has assumed that the elastic force that causes desaccommodation resides in the choroid, since desaccommodation is associated with relaxation of the muscle. This ultrastructural study shows how ciliary muscle is linked via elastic tendons to tissue structures that are responsible for the elasticity of the choroid.

In the region of the posterior attachment of the ciliary muscle, young animals show a connection between the elastic lamina of Bruch's membrane and the basal lamina of the pigmented epithelium. Similar microfibrillar connections between elastic material and the basal lamina of the PE have also been reported for the posterior ciliary body in the bovine eye and in rabbits and humans. In the primate eye, this attachment might be responsible for the move-
Fig. 14A. Oblique-tangential section of Bruch's membrane and the pigmented epithelium in region of the attachment of the ciliary muscle tendons in a 34-year-old monkey. The elastic lamina of Bruch's membrane (asterisks) appears thicker than those in young animals. The homogenous component of the elastic material stains electron lucent. Between the elastic lamina and the pigmented epithelium (PE), a high amount of collagen fibrils in an electron-dense ground substance is seen. The collagen fibrils contact the basal lamina of the PE (arrows) and the elastic lamina. In areas of contact, the elastic lamina shows protrusions (arrowheads). The area of magnification is shown (circle) in Figure 12B (electronmicrograph, X4000).

Fig. 14B. Higher magnification of Figure 12A. Microfibrils (asterisk) emerge from the elastic layer and seem to be in continuity with thicker collagen fibrils, which show their typical periodicity (arrow) (X40,000).

The elastic fibers in the region of the posterior attachment of the ciliary muscle undergo structural age-related changes. These changes are different from those, that have been described in the eye, eg, in the posterior parts of the choroid.43,44,45 We do not know to what extend these changes influence the functional
Fig. 15. Oblique–tangential section of the elastic layer of Bruch’s membrane in the region of the attachment of the ciliary muscle tendons in a 34-year-old monkey. The collagen fibrils, which run between pigmented epithelium and the elastic layer (E) in areas, contact the elastic fibers directly, without intervening microfibrils. At points of contact, osmiophilic material is seen between collagen fibrils and the protrusions of the elastic layer (arrows) (electronmicrograph, x40,000).

properties of the fibers and whether they represent elastic fibers in the true sense of the word. Further studies are needed, eg, using antibody staining, to determine whether these fibers contain elastin.

A distinct feature other than the structural changes in the elastic fibers is the pronounced increase in the amount of microfibrils and collagen fibrils, which are closely associated with the elastic fibers. A mechanical link between elastic and those collagen fibrils is suggested by the presence of osmiophilic points of contact between elastic and collagen fibrils not seen in young animals. The contacts are not affected by acute topical administration of pilocarpine or atropine, which would affect the tension in the tissue. Thus, the fibrils have a mechanical strength. The reason for the age-related increase in fibrillar material in the region of the posterior attachment of the ciliary muscle is not clear. It is possible that mechanical traction of the accommodating muscle serves as a stimulus for the production of extracellular material that accumulates during the lifetime of the animal. It has been shown in vitro that cyclic stretching stimulates synthesis of collagen and other matrix components by arterial smooth muscle cells. Hypertrophy of intestinal smooth muscle in vivo is associated with an increase in the content of collagen. Increase in inelastic collagogenous material and its association with the elastic system might stiffen the posterior insertion of the ciliary muscle with increasing age and contribute to presbyopia. Interestingly, it has been suggested that a three-dimensional interlocking of both collagen and elastic fibers may be the cause of decreased tissue compliance in aged skin.

Age changes in individual ciliary muscle cells are minimal. However, the fact that the area of rhesus monkey ciliary muscle in meridional sections decreases by approximately one-third with age, might indicate loss of contractile muscle cells. Thus, the muscle might not be able to overcome the increased resistance of the posterior attachment. The contractions of the muscle might become more isometric as the muscle is restrained in a relaxed position.

Reports show, using ingenious but indirect methods, that human ciliary muscle does not weaken with onset of presbyopia. However, forces on the lens during accommodation are determined less by the contractile strength of the ciliary muscle than by its position and configuration. Therefore, changes in elasticity of the posterior attachment of the ciliary muscle might also be an important factor that accounts for age-dependent loss of accommodation in humans. However, since differences have been reported in age-related morphologic changes of the ciliary body between humans and rhesus monkeys, further studies are needed to determine whether similar mechanisms affect human presbyopia.

In summary, we hypothesize that an age-related increase in the amount of inelastic fibrillar material causes decreased compliance of the posterior insertion of the ciliary muscle in rhesus monkeys. As a result, the anterior–inward movement of the ciliary muscle during accommodation diminishes with age.
Loss of ciliary muscle movement might be responsible for or at least contribute to presbyopia in primates.

Key words: ciliary smooth muscle, presbyopia, elastic fibers, aging, rhesus monkey

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