The Spatial Aspect and Fine Structure of the Orbital Muscle of the Mouse

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Summary. The spatial aspect of the mouse orbital muscle was studied in serial sections by light microscopy. Then the fine structure of the muscle was studied by light and electron microscopy.

The orbital muscle is a large membranous smooth muscle which is the sole wall dividing the orbital cavity from the temporal and infratemporal cavities and encloses all of the orbital contents in its course. The orbital muscle arises from the upper portion of the orbital bony wall, the trochlear cartilage, the caruncle and the lacrimal canaliculi, and ends in the lower portion of these organs. The anterior margin of the orbital muscle adheres to the levator palpebrae superioris and tarsal muscles, the orbital septum and the inferior orbital margin. The orbital muscle has membranous arising and attaching tendons which contain a large amount of elastic fibers.

The surface membrane of the smooth muscle cells specializes into two kinds of alternate zones; pinocytosis vesicle-rich zones and vesicle-free zones, the latter of which is attached to many myofilaments and come into close contact with the same kind of zones of another adjacent cell to form a desmosome-like junction with a minute gap of about 400Å which contains only one basement lamina. Gap junctions are also often observed between the two smooth muscle cells.

The mouse orbital muscle may correspond not only to the human orbital muscle and periorbital covering the inferior orbital fissure but also to the transverse ligament of Whitnall (1910) and the suspensory ligament of Lockwood (1886). The mouse orbital muscle may function in supporting the orbital contents, especially the eyeball, in regulating blood pressure in the orbital venous sinus and in checking eye movement.

Introduction

The orbital muscle was firstly described in man and mammals by Müller (1858) as a smooth muscle closing off the inferior orbital fissure. He found

that this smooth muscle develops more strongly in mammals than in man. Later the existence of the orbital muscle had been confirmed in man by Ernyei (1934), Wasano (1948), Rohen (1953), Welt and Zacharias (1970) and Vermeij-Keers (1973),

in the rabbit by Nagai (1951), in the rat by Page (1973) and in the mouse by Yamashita (1979). However, the precise spatial aspect of the orbital muscle is obscure, because this muscle is so fragile and has so large an extension that its dissection is difficult, and the fine structure of the orbital muscle has not yet been reported. The purpose of the present study is to clarify the spatial aspect of the mouse orbital muscle in serial sections by light microscopy and its fine structure by light and electron microscopy.

Materials and Methods

Five adult mice, pure strain KH-1 (*Mus wagneri* var. *albula*) were used in this study. Using three mice, serial sections of 30 μ in thickness were prepared for studying the spatial aspect of the orbital muscle, according to procedures described previously (Yamashita, Takahashi and Honjin, 1980). For electron microscopy, small pieces of muscle were fixed in phosphate buffered paraformaldehyde, glutaraldehyde and osmium tetroxide mixture and embedded in Epon 812. Ultrathin sections were stained by uranyl acetate and lead citrate method or tannic acid method for elastic fibers (Kajikawa, Yamaguchi, Katsuda and Miwa, 1975) and examined with an HU-12 or H-500 type electron microscope. Semi-thin sections stained with toluidine blue were used for light microscopy.

Results

I. The Spatial Aspect of the Mouse Orbital Muscle

The mouse orbital muscle is a large membranous smooth muscle which encloses almost all of the orbital contents (Text-figure). Its posterior portion with a small muscle belly can be distinguished

from its anterior portion by a rather large muscle belly.

The posterior portion of the muscle arises from the upper wall of the orbit by its arising tendon (Fig. 1) and runs between the orbital venous sinus and the temporal muscle (Fig. 2). Then it passes between the orbital venous sinus and the lateral pterygoid or masseter muscle and ends in the medial wall of the orbit by its attaching tendon (Fig. 3). In the posterior portion, the orbital muscle has a small muscle belly situated in the lower-lateral edge of the orbit, where the temporal muscle meets the lateral pterygoid and masseter muscles (Fig. 2).

The anterior portion of the orbital muscle arises from the trochlear cartilage, the lacrimal caruncle and the superior lacrimal canaliculus by its arising tendon. It encircles the eyeball just behind the equator and ends in the trochlear cartilage, the caruncle and the inferior lacrimal canaliculus by its attaching tendon. The orbital muscle adheres to the levator palpebrae superioris and tarsal muscles and the orbital septum with the upper and lateral parts of its anterior margin (Fig. 5). The lower part of the anterior portion of the orbital muscle extends over the intraorbital lacrimal gland and attaches to the inferior orbital margin (Fig. 6). The muscle belly of the anterior portion is large and is situated in the lower part of the orbit. Besides this belly, a small group of smooth muscle cells is also observed in the orbital muscle near the lower edge of the trochlear cartilage.

The orbital muscle is perforated by several veins which connect the orbital venous sinus to the venous system of the head (Yamashita, Takahashi and Honjin, 1980). The lacrimal artery, the zygomatic nerve and its branches, the supraorbital artery, the frontal nerve and

Text-figure. A diagrammatic representation of the frontal view of the spatial aspect of the mouse orbital muscle. Lines marked J indicate the muscle(M)-tendon (T) junction of the orbital muscle. Arrows represent running courses of veins (C) connecting the orbital venous sinus with the venous system of the head, the lacrimal artery (L), the zygomatic nerve (Z), the supraorbital artery (S), the frontal nerve (F), and the superior (SO) and inferior (IO) oblique muscles.

the inferior oblique muscle also run through the orbital muscle (Text-figure).

A membranous smooth muscle expands between the lacrimal sac and the masseter muscle. This smooth muscle, which corresponds to the human lacrimal fascia, connects with the lower-medial part of the orbital muscle.

II. The Fine Structure of the Mouse Orbital Muscle

1. Light Microscopy

The muscle belly found in both anterior and posterior portion of the orbital muscle is about 20 to 30 μ in thickness and consists of 5 to 10 layers of smooth muscle cells and a relatively small amount of interstitial connective

tissue. The arising and attaching tendons of the orbital muscle contain a large amount of elastic fibers (Fig. 7). Where the orbital muscle encloses the orbital venous sinus, its muscle belly and tendons come into such close contact with the endothelium of this venous sinus that they appear to be the wall of the orbital venous sinus (Figs. 4 and 8).

Smooth muscle cells in the orbital muscle have a long spindle-like shape with an average length of about $100\ \mu$ and a diameter of about $5\ \mu$ in relaxed condition. Every muscle cell has a long cylindrical nucleus of about $25\ \mu$ in length and about $2\ \mu$ in diameter. In contracted condition the nucleus shows a deep helical indentation.

2. Electron Microscopy

The smooth muscle cells have many pinocytosis vesicles or caveolae, which are distributed in several longitudinal zones of the cell surface. These pinocytosis vesicle-rich zones alternate with pinocytosis vesicle-free zones which have a dense amorphous layer just inside the cell surface. Many myofilaments attach to the dense amorphous layer. These two kinds of zones, vesicle-rich and vesicle-free, face respectively the same kind of zones of another cell to form a pairing of zones (Fig. 9). Between two pinocytosis vesicle-rich zones, there is found a gap of about $900\ \text{\AA}$ in distance containing a small tissue space. The pinocytosis vesicle-free zones come into close contact intervening a minute gap of about $400\ \text{\AA}$ which contains only one dense lamina resulting from fusion of two sheets of basement lamina. Gap junctions or nexuses are also often observed between the two smooth muscle cells (Fig. 10). A few fibrocytes and melanocytes are dispersed among the smooth muscle cells.

Tendons of the orbital muscle are

characterized by its rich content of elastic fibers running in parallel array together with collagen fibrils and a few fibrocytes (Fig. 11 and 12). The elastic fibers have an average diameter of about $1.2\ \mu$ and sometimes show bifurcation. At the muscle-tendon junctions, the elastic fibers often split into minute branches and adhere to the smooth muscle cells side by side at their pinocytosis vesicle-free zones (Fig. 13 and 14). Often the junctions are formed at the surface grooves of the smooth muscle cells as shown in Figure 13.

Discussion

Based on the comparative anatomy of the vertebrate orbit, Burkard (1902) described that the periorbita of all vertebrates encloses all of the orbital contents. According to him, the periorbita of cartilaginous fishes is composed of connective tissue rich in elastic fibers. The periorbita of bony fishes is composed of connective tissue rich in smooth muscle cells and elastic fibers. The periorbita of frogs and birds is composed of skeletal muscle derived from the muscles of mastication. The periorbita of reptiles contains a large number of smooth muscle cells. In mammals the more completely the orbital cavity is surrounded with bony wall, the more regressive the periorbita becomes. Müller (1858), Rohen (1953) and Welt and Zacharias (1970) noted the intimate relationship between the orbital muscle and the periorbita in man and regarded the periorbita adjacent to the orbital muscle as its elastic tendon.

The present study indicates that the mouse orbital muscle has arising and attaching tendons which contain a large amount of elastic fibers, similar to the mouse tarsal muscle reported by Yamashita (1979). In the mouse the orbital

muscle together with its elastic tendon is synonymous with the periorbita named by Burkard (1902). The posterior portion of the mouse orbital muscle may correspond to the human orbital muscle and periorbita extending across the inferior orbital fissure and the infra-orbital groove. The anterior portion of the mouse orbital muscle may correspond to the transverse ligament of Whitnall (1910) and the suspensory ligament of Lockwood (1886), which join at the medial and lateral edges of the orbit and are also designated by Fink (1957) as the superior and inferior transverse fascial expansions, respectively.

The present study indicates that the fine structure of the mouse orbital muscle is similar to that of the mouse tarsal muscle reported by Yamashita (1979). The surface membrane of smooth muscle cells specializes mainly into two kinds of alternately located zones; pinocytosis vesicle-rich zones and vesicle-free zones, the latter of which shows the myofilament attaching to the surface. These two kinds of zones tend to pair with the same kind of zones of the adjacent cells.

The pinocytosis vesicles of smooth muscle cells are thought to be analogous to T tubules of striated muscle fibers by Gabella (1971) and Devine, Simpson and Bertaud (1971), and to be calcium extrusion pumps by Popescu, Diclescu, Zelck and Ionescu (1974). The electron dense zones free from pinocytosis vesicles of smooth muscle cells in the artery are thought by Pease and Morinari (1960) to be the site where myofilaments are anchored. Devine, Simpson and Bertaud (1971) reported in the guinea-pig artery and vas deferens that the pinocytosis vesicle-free zones are arranged in longitudinal banding pattern alternating with pinocytosis vesicle-rich zones. In the present study, the pinocytosis vesicle-

free zones of adjoining muscle cells come into close contact with each other to form desmosome-like junctions with a minute gap of about 400 Å which contains only one basement lamina.

In the orbital muscle, the gap junctions are well developed between the two smooth muscle cells as compared with the mouse tarsal muscle (Yamashita, 1979). The gap junctions are thought by Evans and Evans (1964) to play an important role in evoking rhythmical contraction by allowing the passage of excitation from one smooth muscle cell to another.

In the present study, electron microscopy revealed the intimate contact of elastic fibers to smooth muscle cells at the muscle-tendon junctions. Such intimate contact of elastic fibers to smooth muscle cells has been confirmed by electron microscopy in the cat and monkey arteries by Pease and Morinari (1960) and in the mouse tarsal muscle by Yamashita (1979).

The orbital muscle closes off the inferior orbital fissure in man and other mammals. Especially in the mouse, this muscle encloses almost all of the orbital contents and is the sole wall dividing the orbital cavity from the temporal and infratemporal cavities. The muscle belly of the posterior portion of the mouse orbital muscle is situated at the weak region where the temporal muscle meets the lateral pterygoid and masseter muscles. The rather stout muscle belly of the anterior portion of the mouse orbital muscle is situated in the lower part of the orbit just behind the bulbar equator. These findings suggest that the mouse orbital muscle functions as a supporting fascia to prevent the orbital contents from herniating and to suspend the eyeball as the human suspensory ligament of Lockwood (1886) does.

The mouse orbital muscle covers most of the external surface of the orbital

venous sinus, so that contraction of this muscle may induce the increase of blood pressure in this sinus resulting in protrusion of the eyeball and excretion from the Harder's gland as we suggested in a previous paper (Yamashita, Takahashi and Honjin, 1980). Moreover the mouse orbital muscle may directly protrude the eyeball by constricting the eyeball just behind its equator with the anterior portion of this muscle. The anterior portion of the mouse orbital muscle may also play a role in checking eye movement, as the human superior and inferior transverse fascial expansions of Fink (1957) do.

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Explanation of Figures

Figures 1~7 are photomicrographs and Figures 8~14 are electron micrographs.

Plate I

- Fig. 1, 2 and 3. A frontal section of the mouse orbit. The arising tendon (t), the muscle belly (m) and the attaching tendon (t) are observed in Figures 1, 2 and 3, respectively. H: Harder's gland. Resorcin-fuchsin stain. $\times 40$.
- Fig. 4. The orbital venous sinus (v) extending between the orbital muscle (o) and the Harder's gland (H). Toluidine blue stain. $\times 400$.
- Fig. 5 and 6. A sagittal section of the mouse orbit. The upper and lower parts of the anterior portion of the orbital muscle (o) are observed in Figures 5 and 6, respectively. H: Harder's gland, i: intraorbital lacrimal gland, l: levator palpebrae superioris muscle, ma: masseter muscle, s: orbital septum, v: orbital venous sinus. Resorcin-fuchsin stain. $\times 40$.
- Fig. 7. A longitudinal section of the orbital muscle. The muscle(m)-tendon (t) junction is observed. H: Harder's gland. Resorcin-fuchsin stain. $\times 700$.

Plate II

Fig. 8. The orbital venous sinus (v) extending between the orbital muscle and the Harder's gland (H) as shown in Figure 4. ns: a smooth muscle cell of the orbital muscle. $\times 7,500$.

Fig. 9. A longitudinal section of smooth muscle cells of the orbital muscle. Note pinocytosis vesicle-rich (r) and vesicle-free (f) zones pairing with the same kind of zones of the adjacent cells. $\times 30,000$.

Plate III

Fig. 10. A transverse section of smooth muscle cells of the orbital muscle. Note gap junctions (g) between the two smooth muscle cells. $\times 30,000$.

Fig. 11 and 12. Longitudinal sections of the tendon of the orbital muscle. Elastic fibers (e) are hardly stained by uranyl acetate and lead citrate method as shown in Figure 11, but deeply stained by tannic acid method as shown in Figure 12. $\times 15,000$.

Plate IV

Fig. 13 and 14. Transverse and longitudinal sections of the muscle-tendon junction of the orbital muscle, respectively. e: elastic fibers, ns: smooth muscle cells. $\times 15,000$.