ARCHITECTURE AND MUSCLE FIBER TYPES OF LAMPREY MYOTOME

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ABSTRACT

In myotome muscle fibers composing the trunk of the lamprey (*Lampetra japonica*), parietal muscle fibers and central muscle fibers, which are histochemically and functionally different from each other, formed single compartments which then formed multiple units. Parietal muscle fibers arranged like a chain in the lateralis, were histochemically identified as red muscle fibers, and classified as slow contracting fibers (Type 1). In contrast, central muscle fibers were present in the medialis surrounded by parietal muscle fibers and histochemically were fast contracting white muscle fibers (Type 2). These observations of the architecture of muscle fibers and muscle fiber types suggest that red muscle fibers (parietal muscle fibers) forming the lateralis of the lamprey are involved in trunk architecture because of their continuous tonic contraction, and white muscle fibers (central muscle fibers) located in the medialis function act as a propelling force used in swimming.

The skeletal muscle of a vertebrate is composed of red muscle fibers (Type 1), white muscle fibers (Type 2) and intermediate muscle fibers (Type 3) (12, 21, 30). The muscle fiber types show constant localization and distribution in one skeletal muscle (10, 24). In lamprey, however, which is an agnathia included as a member of the vertebrate phyla, muscle fibers forming the trunk are composed of two specific types, parietal muscle fibers and central muscle fibers (23). The architecture of the muscle fibers and differentiation of muscle fiber types were histochemically assessed in this species with light microscope, scanning electron microscope (SEM) and transmission electron microscope (TEM). The relationship of these muscle types to the locomotion system used for swimming is discussed.

MATERIALS AND METHODS

Preparation of Muscle Tissue

The materials were transverse sections of the abdomen of 5 adult lampreys (*Lampetra japonica*) whose total length was about 55 cm. The excised muscle tissue block was frozen with liquid carbon dioxide

immediately after excision, and thinly sliced in 20–25 μm sections with a Sartorius-type microtome. The sections were then stained with hematoxylin and eosin (HE) and the muscle fiber types were classified according to the succinic dehydrogenase (SDH) method of Nachlas *et al.* (17). In doing so, muscle fiber activity with marked formation of formazan was regarded as red muscle fiber or Type 1, while inactive muscle fiber with weak formation of formazan was considered white muscle fiber or Type 2. According to the method of Davis and Gunn (4) using Sudan black B (SBB) staining, the muscle fiber staining strongly positive with SBB was regarded as red (Type 1), while that which was not stained with SBB as white (Type 2).

Determination of Muscle Fiber Size

By means of a mechanical stage attached to the light microscope and a visualizer, 100 cross-sectional areas of each parietal (red muscle type) and central muscle fiber (white muscle type) were directly identified from each specimen, and their sizes were calculated in μm^2 equivalents using an image ana-

Fig. 1 Transverse view of the lamprey abdomen. My, myotome; Ms, myoseptum; Nc, spinal cord; Df, dorsal fin

lyzer connected to a computer (Wacom XY Digitizer, WT 440SE, CPU; NEC-9801). The mean (M), standard deviation (SD) and coefficient of variation (CV) were integrated.

Specimens for SEM

The muscle tissue blocks excised from the abdomen of the immobilized lampreys were first immersed in 12.5% and then in 25% dimethyl sulfoxide (DMSO) for 1 h each. Subsequently, they were placed in 50% DMSO, frozen with liquid carbon dioxide, and cut in frontal, sagittal and horizontal planes. After washing in a buffered solution, the specimens were immersed in a solution of 0.1% osmic acid at room temperature for 3 days. They were then treated by conductive staining according to the tannic acid osmium method, dehydrated in a graded series of ethanol, placed in isoamyl acetate

and critical point dried with liquid carbon dioxide. The surface of the specimen was examined with a field emission type scanning electron microscope (S-700; accelerating voltage, 20 kv).

Specimens for TEM

Immediately after the lampreys were decapitated, the skeletal muscle was removed, and fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, at 4°C for 2 h, and post-fixed in 0.1% OsO₄ in 0.1 M cacodylate buffer, pH 7.2, at 4°C for 2 h. The specimens were dehydrated in a graded series of ethanol and embedded in Epon 812 through propylene oxide. Ultrathin sections (100 nm) for conventional electron microscopic observation were serially cut with an MT-2B (Dupont, Sorvall, U.S.A.) ultramicrotome, and placed on 150-mesh copper grids. They were then stained with 4.0% uranyl acetate in 50% ethanol for 5 min, and Reynold's lead citrate (2.66%) for 5 min, before being examined with a transmission electron microscope (JEM 1200 EX Mark-II) at an accelerating voltage of 80 kv.

RESULTS

Histological Findings of Muscle

Fig. 1 shows a tissue specimen in transverse section from the lamprey abdomen. The myotome layer ran from the dorsal to the ventral sides along the long axis of the trunk, and were composed of 4 to 5 layers divided in the superficial layer on the dermal side (Fig. 2). In the subcutaneous mucous secretory layer, transverse images of muscle fibers were orientated toward myosepta in the deep tissue of the exterior wall on both sides. They were arranged like a chain at constant intervals, and were ovoid in form. In the medialis, more muscle fibers were present and formed a specific compartmental architecture. Fig. 3 shows a view of muscle fibers in the lateralis (P) and medialis (C). Two types of muscle fibers with distinctly different sarcoplasmic contents were observed. Those in the lateralis (P) were lightly stained by HE, the terminal coupling between the muscle fibers was strong, and the nucleus was located in many cases subsarcolemmally. In contrast, the muscle fibers in the medialis (C) were deeply stained by HE and were different from those in the lateralis and displayed an internal nucleus. The coupling between muscle fibers was weak, and terminal coupling disappeared during preparation of the specimens.

Fig. 2 Higher magnification of Fig. 1 in proximity to the dorsum. Muscle fibers forming the right and left lateralis (parietal muscle fibers) and muscle fibers in the medialis (central muscle fibers) from each compartment. Hematoxylin-eosin staining. $\times 100$. Ep, epidermis; Fd, fibrous dermis; p, parietal muscle fiber; C, central muscle fibers

Fig. 3 Higher magnification of Fig. 2, showing parietal muscle fibers (P) and central muscle fibers (C). Hematoxylin-eosin staining. $\times 200$

Muscle Fiber Types

By using mitochondria of parietal muscle fibers in the lateralis and central muscle fibers in the medialis of the lamprey myotome as indicators, the fibers were classified into muscle fiber types with SDH activity (Figs. 4 and 5). The parietal muscle fibers (R) located in the lateralis were of the active type and showed strong formation of formazan, while the central muscle fibers (W) were of the inactive type and displayed no formazan forming activity. Muscle fibers were classified into fiber types by SBB staining using sarcoplasmic lipids and phospholipid bound to the mitochondrial matrix. As illustrated in Figs. 6 and 7, muscle fibers in the

medialis (W) showed no pigmentary reaction, while the muscle fibers in the lateralis (R) were strongly stained with pigment in the same way as with SDH. The staining pattern of the parietal and central muscle fibers with SBB and SDH were similar (Figs. 5 and 7) leading us to conclude that Type 1 red muscle fibers were slow contracting and the central muscle fibers in the medialis were fast contracting Type 2 white muscle fibers.

Determination of Muscle Fiber

The number of muscle fibers in one compartment, muscle fiber size and variation in the size (CV) of parietal muscle fibers (red muscle type) and central

Fig. 4 Muscle fiber type in the lamprey myotome treated with succinic dehydrogenase. Parietal muscle fibers (R) show positive reaction (Type 1 red muscle), whereas central muscles fibers (W) are negative (Type 2 white muscle). Transactional image. $\times 100$

Fig. 5 Higher magnification of Fig. 4. R, red muscle fiber (parietal muscle fiber); W, white muscle fiber (central muscle fiber). $\times 400$

Table 1 *Muscle Fibers of Lamprey Myotome*

	Number of muscle fiber in 1 compartment	Size of muscle fiber (μm^2) M $\pm$ SD	Coefficient of variation SD/M $\times 100$
Parietal muscle fiber	21–25	636.3 $\pm$ 166.6	26.2
Central muscle fiber	28–30	568.6 $\pm$ 176.7	31.0

muscle fibers (white muscle type) were determined on the basis of the mean value for 5 lampreys (Table 1). Each compartment was composed of 21–25 parietal muscle fibers and 28–30 central muscle fibers. Parietal muscle fibers were as thick as $636.3 \pm 166.6 \mu\text{m}^2$ and central muscle fibers $568.6 \pm 176.7 \mu\text{m}^2$. Thus, parietal muscle fibers

(red muscle type) were larger than central muscle fibers by about 1.1 times. Variation in thickness between these two muscle types was determined from the coefficient of variation (CV: $\text{M}/\text{SD} \times 100$) and showed parietal muscle fibers to be more homogeneous at 26.2, while central muscle fibers showed a tendency towards variance at 31.0.

Fig. 6 Muscle fiber type of the lamprey myotome stained with Sudan black B. The staining pattern of the central muscle fibers (W) corresponds to that with succinic dehydrogenase in that the central muscle fibers do not show any pigmentary reaction, whereas parietal muscle fibers (R) are deeply stained with Sudan black B. $\times 100$

Fig. 7 Higher magnification of Fig. 6. R, red muscle fiber (parietal muscle fiber); W, white muscle fiber (central muscle fiber). $\times 400$

Electron Microscopic Findings

Figs. 8 and 9 illustrate the SEM findings of the muscle fibers in a myotome, obtained by cutting

the lamprey abdomen transversely. Central muscle fibers were present adjacent to the parietal muscle fibers which continued from the myoseptum, suggesting that these two muscle fibers formed many

Fig. 8 SEM finding of a lamprey myotome showing central muscle fibers (c) between the upper and lower layers of the parietal muscle fibers (P) originating in the myoseptum (My).

Fig. 9 Higher magnification of Fig. 8 illustrating the adjacent parietal muscle fibers (P), which display capillary vessels (Ca) in the space between the two layers of muscle fibers.

units. Capillary blood vessels (Ca) ran between the two layers. The two types of muscle fibers are illustrated stereoscopically in Fig. 10 and show how the parietal muscle fibers (P) are entangled with the central muscle fibers (C). Using TEM, many bulb-

ous rod mitochondria (Mi) with well-developed cristae were localized subsarcolemmally (Sar), and large lipid droplets (L) were present among the mitochondria (Figs. 11 and 12). Small-sized mitochondria were in proximity to the I band, with sar-

Fig. 10 SEM view showing entanglement of the parietal muscle fibers (P) and central muscle fibers (C)

coplasmic reticula (Sr) surrounding muscle fibrils like a network. These findings were characteristics of red muscle type. In contrast, in the central muscle fibers, a near absence of mitochondria was observed (Fig. 13) and the number of sarcoplasmic reticula was smaller than in parietal muscle fibers. Nucleoli of the internal nucleus in the sarcoplasm were distinctly visible, and various wandering cells were present in the myoseptum. The muscle fibers were surrounded by segmentary sarcoplasmic reticula. No lipid droplets of mitochondria were present in proximity to the subsarcolemma (Fig. 14). These findings were characteristic of the white muscle type.

DISCUSSION

Agnathia cyclostomata petromyzonidae along with myxinidae are regarded as the most primitive species of the vertebrate (9, 13, 26). For this reason, this species is a noteworthy model for the phylogenetic study of archetype regarding the functional differentiation of cellular tissue. The present study used lampreys (*Lamprey japonica*) to examine the architecture of the myotome, i.e., the rudiment of skeletal muscle. Although myotomes are formed from mesoderm, as is observed in one fetal stage of higher vertebrates, the developmental course is

occasionally difficult to be adequately observed because of its rapidity.

In contrast, the lamprey myotome is distinctly observable until the end of its life. Hence, histological assessment has revealed specific morphological findings of compartments composed of a red muscle type, i.e., slow contracting parietal muscle fiber and a white muscle type, i.e., fast contracting central muscle fibers. This architecture of muscle fibers in the lamprey myotome is unlike the architecture of the three types of muscle fibers in fish, amphibians, reptiles, birds and mammals (1, 6, 8, 20).

HE staining showed strong coupling among parietal muscle fibers in the lamprey lateralis, whose nuclei were localized subsarcolemmally, and weak terminal coupling among central muscle fibers in the medialis, which were individually separated during preparation of the specimens (Figs. 2 and 3). Nakao (18, 19) has also studied terminal coupling in the lamprey by electron microscopy. There have been other reports describing the details of the triad between the sarcoplasm and perimysium, and of peripheral coupling as compared to interior coupling. The nuclei of central muscle fibers showed an internal nucleus (as shown in Figs. 3 and 13) similar to amphibians and reptiles (12). These fibers were also more apocytic than were

Fig. 11 TEM view of lamprey parietal muscle fibers with many mitochondria and lipid droplets. Sr, sarcoplasmic reticulum; Sar, sarcolemma

Fig. 12 TEM view of lamprey parietal muscle fibers illustrating subsarcolemmal (Sar), mitochondria (Mi) and lipid droplets (L) in the sarcoplasm

parietal muscle fibers. This suggests a difference in metabolic activity between these two types of muscle fibers. Muscle fibers of higher vertebrates are classified into type 1 red muscle fibers, type 2 white muscle fibers and type 3 intermediate muscle fibers (25, 28, 32), a finding which has been clarified by electron microscopy (7, 22, 27, 31).

In lamprey, however, which is the lowest of the existing vertebrates, parietal muscle and central muscle fibers correspond to the red and white muscle fibers of higher vertebrates, respectively, as described by Meyer (15) using oxidative enzymes and myosin-ATPase. Our present study, using SDH and SBB, confirmed these findings. Thus, the lam-

Fig. 13 Lamprey central muscle fibers (white muscle fibers) as viewed by TEM in which the deficiency of mitochondria and lipid droplets can be seen in the sarco-plasm, as well as the distinct presence of nucleoli (Nu), an internal nucleus (In), a small number of wandering cell (Wc), and connective tissue.

Fig. 14 TEM view of lamprey central muscle fibers (white muscle fiber) showing the subsarcolemmal (Sar) deficiency of mitochondria and lipid droplets

prey muscle fibers were composed of only two types of muscle fibers (2, 29), and the intermediate muscle fiber has not differentiated in the primitive agnathia cyclostomata petromyzonidae (3, 5, 16). In the Atlantic hagfish, i.e., the same cyclostomata

myxinidae, intermediate muscle fibers have been identified by Korneliussen and Nicolaysen (11) and Melligren and Mathisen (14). Despite the fact that they are included in the same agnathia class, there is a difference in functional differentiation of

muscle fibers in terms of evolutionary stage. Parietal muscle fibers in the lamprey had many mitochondria, as shown by electron microscopy (Fig. 11) and by the positive SDH reaction (Figs. 4 and 5). Simultaneously, chromatophilic lipid droplets as defined by SBB and were released in the form of phospholipids on the laminar surface of cristae and matrices of mitochondria and in the sarcoplasm were found (Figs. 6 and 7). In contrast, central muscle fibers had no mitochondria in their sarcoplasm (Fig. 12). As a result, the central muscle fibers had an S pattern as observed by light microscopy (Figs. 5 and 7). The specific architecture of muscle fibers in the lamprey suggests their role in locomotion. The fact that parietal muscle fibers (R) in the lateralis, i.e., red muscle fibers, were arranged in an ovoid pattern and formed many compartments, implies that the morphology is appropriate for maintaining the body cavity and trunk against exterior pressure by tonic contraction, since differentiation stopped in the notochord stage and the vertebral column never developed. In contrast, central muscle fibers (W) in the medialis, i.e., white muscle fibers, are suggested to have a kinetic role and to function as a propelling force in swimming of the lamprey.

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