Histochemical Properties of Myofiber Types and Lack of Muscle Spindles in the Caudal Pharyngeal Constrictor Muscles of Sheep

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Abstract The purpose of the present study was to examine histochemical properties of myofiber types and presence or absence of muscle spindles in the caudal pharyngeal constrictor (thyropharyngeus and cricopharyngeus) muscles of sheep. Unfixed muscle sections were stained with myosin ATPase (preincubation pH 4.3 and 10.5), reduced nicotinamido adenine dinucleotide dehydrogenase, and 3-hydroxybutyrate dehydrogenase (3-HBD). Myofiber types were classified by the differences of the histochemical properties. The thyropharyngeus muscle had 12% type I, 88% type IIA, and no type IIB myofibers. The cricopharyngeus muscle possessed 53% type I, 47% type IIA, and no type IIB myofibers. The myofiber types of the thyropharyngeus and cricopharyngeus muscles were small in diameter. Part of type I myofibers showed a strong 3-HBD activity, whereas all type IIA myofibers showed no 3-HBD activity in the two muscles. No muscle spindle was found in the two muscles. The thyropharyngeus and cricopharyngeus muscles composed of types I and IIA myofibers seem to be adapted to close the pharyngeal cavity repeatedly for the rumination.

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The pharyngeal constrictor muscles, which include the rostral, middle, and caudal muscle groups, transfer a bolus of food from the oral cavity through the pharynx into the esophagus during deglutition, and from the esophagus through the pharynx into the oral cavity during regurgitation in the ruminants. The caudal pharyngeal constrictor muscles, which are divided into the thyropharyngeus and cricopharyngeus muscles, are situated in the laryngopharynx. These muscles are primarily for the deglutition, forcing the bolus into the esophagus by sequential contraction. The cricopharyngeus muscle of humans has many type I myofibers^{2,4)}, but the cricopharyngeus muscle of guinea pigs has few type I myofibers¹⁾. No muscle spindle has been found in these muscles^{1,2,4)}, although

the skeletal muscles have muscle spindles which are proprioceptive receptors sensitive to changes in muscle length.

Ruminants spend much time a day in grazing and ruminating. The ovine masseter muscle which is adapted to long-term mastication for grazing and rumination has 100% type I myofibers with very strong 3-hydroxybutyrate dehydrogenase activity¹⁰⁾. The thyropharyngeus and cricopharyngeus muscles need to contract repeatedly during grazing and rumination. The ovine thyropharyngeus and cricopharyngeus muscles are assumed to possess histochemical properties adapted to repeated contraction. Whether the muscle spindle exists in the caudal pharyngeal constrictor muscles of the sheep is unclear.

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The purpose of the present study was to examine the histochemical characteristics and composition of myofiber types of the ovine thyropharyngeus and cricopharyngeus muscles. Also, the presence or absence of muscle spindles was studied in the two muscles.

Materials and Methods

Five adult female sheep (Corriedale × Suffolk; weight 48–64 kg) were used in this study. After slaughter, muscle samples were removed from the thyropharyngeus and cricopharyngeus muscles. Transverse sections (1 cm thick) were taken from the belly of muscles, and were frozen in a mixture of acetone and dry ice. Cross sections (10 μm thick) were cut serially on a cryostat and mounted on microslides. Unfixed muscle sections were stained with myosin ATPase reactivity after preincubation at pH 4.3 and pH 10.5^{3,10}, reduced nicotinamido adenine dinucleotide (NADH) dehydrogenase^{7,10}, and 3-hydroxybutyrate dehydrogenase (3-HBD)¹⁰. Myofibers were classified into type I, IIA, and IIB myofibers by differences in the histochemical activities of myosin ATPase and NADH dehydrogenase¹¹. Diameters of myofibers were obtained by measuring their smallest dimension with an ocular micrometer. *t*-test and Tukey's multiple comparison method were used for statistical analysis, and differences between means were considered significant at $P < 0.05$.

Results

Type I myofibers were strongly reactive for myosin ATPase after acid preincubation and unreactive for myosin ATPase after alkaline preincubation and stained strongly with NADH dehydrogenase. Type IIA myofibers were unreactive for myosin ATPase after acid preincubation and strongly reactive for myosin ATPase after alkaline preincubation and stained moderately to strongly with NADH dehydrogenase. No type IIB myofibers that showed the same myosin ATPase reactivities as type IIA myofibers and weak activity of the NADH dehydrogenase were found in the thyropharyngeus and cricopharyngeus muscles. The thyropharyngeus muscle had many type IIA myofibers (Table 1, Fig. 1

Table 1. Percentages of myofiber types in the caudal pharyngeal constrictor muscles of sheep

Muscles	Myofiber type		
	I	IIA	IIB
Thyropharyngeus	12.2 ± 2.9 ^b	87.8 ± 2.9 ^c	0 ^a
Cricopharyngeus	52.6 ± 4.4 ^b	47.4 ± 4.4 ^b	0 ^a

Mean ± Standard deviation (n = 5)

^{a-c} Significant differences between different superscripts in same muscle ($P < 0.05$).

a-c). On the other hand, in the cricopharyngeus muscle, the percentage of type I myofibers was similar to that of type IIA myofibers (Table 1, Fig. 2a-c). The caudal region had more type I myofibers than did the rostral region in the caudal pharyngeal constrictor muscles. The myofiber types of the thyropharyngeus and cricopharyngeus muscles were small in diameter (Table 2). In the thyropharyngeus and cricopharyngeus muscle, part of type I myofibers showed a strong 3-HBD activity, whereas all type IIA myofibers showed no 3-HBD activity (Figs. 1d, 2d). The type I myofibers with strong 3-HBD activity were more numerous in the cricopharyngeus muscle than in the thyropharyngeus muscle.

No muscle spindle was observed in the thyropharyngeus and cricopharyngeus muscles.

Discussion

Histochemical properties of myofiber types

The thyropharyngeus and cricopharyngeus muscles composed of types I and IIA myofibers are assumed to be adapted to close the pharyngeal cavity repeatedly for the rumination in the sheep. Type I and IIA myofibers are resistant to fatigue^{5,8}. The ovine thyropharyngeus muscle with many type IIA myofibers appears to have a greater capacity to transfer the bolus quickly from the pharynx to the esophagus or oral cavity than does the cricopharyngeus muscle because type IIA myofibers contract fast^{5,8}. The thyropharyngeus muscle must constrict the caudal pharynx quickly to prevent the bolus from a drop into the larynx and to force the bolus into the esophagus during deglutition or into the oral cavity during regurgitation.

Myofiber Types in Ovine Pharyngeal Muscles

Figs. 1 and 2. Myofiber types of the thyropharyngeus and cricopharyngeus muscles of sheep. Fig. 1. The thyropharyngeus muscle. Fig. 2. The cricopharyngeus muscle. Figs. 1a and 2a. Myosin ATPase reaction after acid preincubation. Type I myofibers (I) are strongly reactive and type IIA myofibers (A) are unreactive. Figs. 1b and 2b. Myosin ATPase reaction after alkaline preincubation. Type IIA myofibers are strongly reactive and type I myofibers are unreactive. Figs. 1c and 2c. NADH dehydrogenase activity. Type I and IIA myofibers show strong to moderate NADH dehydrogenase activity. No type IIB myofibers, which indicate weak NADH dehydrogenase activity, exist in the two muscles. Figs. 1d and 2d. 3-hydroxybutyrate dehydrogenase (3-HBD) activity. Part of type I myofibers show strong activity of 3-HBD, but type IIA myofibers exhibit no 3-HBD activity. Arrowheads in Fig. 2a indicate type I myofibers with strong 3-HBD activity. $\times 200$.

Table 2. Diameters (μm) of myofiber types in the caudal pharyngeal constrictor muscles of sheep

Muscles	Myofiber type	
	I	IIA
Thyropharyngeus	23.9 $\pm$ 2.9	26.6 $\pm$ 1.0
Cricopharyngeus	24.0 $\pm$ 2.3	22.8 $\pm$ 2.0

Mean $\pm$ Standard deviation (n=5)

The ovine cricopharyngeus muscle possessing 53% type I myofibers seems to be adapted for a sphincter of the pharyngoesophageal junction involved in sustained and repeated constriction. Type I myofibers in the cricopharyngeus muscle in the sheep were larger in proportion than that in the guinea pig¹⁾ and were smaller in proportion than that in the human^{2,4)}. Eighty-two to 86% type I myofibers in the human cricopharyngeus muscle^{2,4)} is presumed to function in long-term constriction of the pharynx. The human cricopharyngeus muscle maintains tonic contraction to prevent from swallowing air⁴⁾.

The myofiber types of the thyropharyngeus and cricopharyngeus muscles were smaller in diameter than those of the muscles of the pectoral girdle¹²⁾ and hindlimb¹¹⁾ in the sheep. The fact suggests that the force of muscular contraction in the constriction of the pharynx is weaker than that in the locomotion or postural maintenance.

In the sheep, the masseter muscle is composed of type I myofibers with strong 3-HBD activity for adaptation to long-term rumination or grazing¹⁰⁾. The ovine cricopharyngeus muscle containing many type I myofibers with strong 3-HBD activity is presumed to be well adapted for long-term repeated constriction of pharynx, because the cricopharyngeus muscle functions as the sphincter of the pharyngoesophageal junction where a bolus passes frequently during the rumination.

Muscle spindles

A muscle spindle is a proprioceptive receptor sensitive to changes in muscle length, and generally has an important role in a motor control of muscular movement via a myotatic reflex. The ovine thyropharyngeus and cricopharyngeus muscles that have no muscle spindle appear to be under the motor control

system without muscle spindles. No muscle spindle has been found in the sphincter muscles such as the human²⁾ and guinea pig¹⁾ cricopharyngeus muscles and striated muscle of the esophagus in the mouse⁹⁾. Krier *et al.*⁶⁾ have suggested that the motor control of sphincter muscles is fit for the maintenance of muscle tone rather than muscle length and that the muscle spindle is unnecessary for the sphincter muscles because the reflex feedback is provided by mucosal mechanoreceptors. When the deglutition occurs, the muscular action of the pharyngeal muscles is controlled by a centrally programmed motor sequence arising from the activity of hindbrain neurons belonging to the swallowing center¹³⁾. These suggest that lack of muscle spindle in the pharyngeal muscles reflects the differences of motor control system between the pharyngeal muscles and the locomotory muscles.

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