

Research Article

Contractile Properties of Esophageal Striated Muscle: Comparison with Cardiac and Skeletal Muscles in Rats

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The external muscle layer of the mammalian esophagus consists of striated muscles. We investigated the contractile properties of esophageal striated muscle by comparison with those of skeletal and cardiac muscles. Electrical field stimulation with single pulses evoked twitch-like contractile responses in esophageal muscle, similar to those in skeletal muscle in duration and similar to those in cardiac muscle in amplitude. The contractions of esophageal muscle were not affected by an inhibitor of gap junctions. Contractile responses induced by high potassium or caffeine in esophageal muscle were analogous to those in skeletal muscle. High-frequency stimulation induced a transient summation of contractions followed by sustained contractions with amplitudes similar to those of twitch-like contractions, although a large summation was observed in skeletal muscle. The results demonstrate that esophageal muscle has properties similar but not identical to those of skeletal muscle and that some specific properties may be beneficial for esophageal peristalsis.

1. Introduction

The external muscle layer of the mammalian esophagus consists of striated muscle fibers, which extend from the pharyngoesophageal junction to the thoracic or abdominal portion [1–3]. This is in contrast to the tunica muscularis of the stomach, small intestine, and large intestine which are constituted entirely of smooth muscle. In humans and cats, the upper and lower portions of the esophagus are composed of striated and smooth muscle fibers, respectively, with mixed composition of these fibers in the middle portion. In dogs, ruminants, and rodents including mice, rats, and hamsters, the muscle layer of the esophagus consists largely of striated muscle fibers. These striated muscle fibers were hitherto considered as “classical” skeletal muscle fibers, innervated exclusively by excitatory vagal efferents that arise from motor neurons originating in the nucleus ambiguus and terminate on motor endplates [4–6].

The major function of the esophagus is propulsion of swallowed food into the stomach by peristalsis [7]. To accomplish effective peristalsis, contraction of each muscle needs to be well coordinated [8, 9]. The peristaltic motility of small and large intestines is mainly regulated by the intrinsic nervous system, which consists of intrinsic sensory neurons, interneurons, and motor neurons that project into the smooth muscle layers [8, 10–12]. In contrast, it has been shown that peristalsis in the esophageal “skeletal” muscle is controlled by the swallowing center in the medulla oblongata through a mediation of extrinsic vagus nerves [7, 13–16]. However, recent studies have revealed that intrinsic neurons also play roles in regulating the motility of the esophageal striated muscle. Morphological studies have shown that esophageal striated muscle receives dual innervation from both vagal motor fibers originating in the brainstem and varicose intrinsic nerve fibers originating in the myenteric plexus, which is called “enteric coinnervation” of esophageal motor endplates [3, 17]. We have also provided evidence that

a local neural reflex pathway in the esophagus, consisting of primary afferent neurons and myenteric neurons, can modulate motility of the striated muscle portion of the esophagus [18–21]. Thus, both extrinsic and intrinsic neural regulations would be important in coordinating the peristaltic motility of the esophagus.

In addition to the neural mechanisms, contractile properties of smooth muscles are beneficial in coordinating motility of small and large intestines. For instance, relatively long duration time of each contraction enables summation of contractions, which in turn results in effective compression of the intraluminal space [22–24]. Furthermore, coupling of neighboring cells by gap junctions would be essential to adjust the timing of muscle contractions [9]. On the other hand, skeletal muscle fibers are insulated from each other [25] and thus may be unfavorable for coordinated motility. To compensate the disadvantages of striated muscle, cardiac muscle possesses several specific properties including expression of gap junctions [26]. We speculated that the striated muscle of the esophagus also has specific properties to establish coordinated peristaltic motility.

Hence, the aim of the present study was to clarify the contractile properties of esophageal muscle with focus on the similarities to cardiac muscle. For this purpose, we compared the mechanical responses of isolated preparations from the esophagus, soleus muscle, and heart in rats.

2. Materials and Methods

2.1. Animals. Male Wistar rats (*Rattus norvegicus*, 12 weeks of age and weighing 200–400 g) were used for the experiments. They were maintained in plastic cages at $22 \pm 2^\circ\text{C}$ with a 12:12 h light-dark cycle (light on 07:00–19:00 h) and given free access to laboratory chow and water. The experiments were approved by the Animal Care and Use Committee of Gifu University.

2.2. Solutions and Drugs. During experiments, tissues were maintained in Krebs' solution consisting of (mM): NaCl 118.4, KCl 4.7, CaCl_2 2.5, MgSO_4 1.2, KH_2PO_4 1.2, NaHCO_3 25, and glucose 11.7. High potassium Krebs' solution consisting of NaCl 4.7, KCl 118.4, CaCl_2 2.5, MgSO_4 1.2, KH_2PO_4 1.2, NaHCO_3 25, and glucose 11.7 was used for inducing high potassium contracture. Tetrodotoxin was used as a blocker of voltage-dependent sodium channels on striated muscle cells. Halothane was used as an inhibitor of gap junctions [27]. Caffeine was used as a releaser of calcium from intracellular stores. Tetrodotoxin, halothane, and caffeine were obtained from Sigma-Aldrich (St. Louis, MO, USA). Tetrodotoxin was initially dissolved in citrate buffer and then dissolved in Krebs' solution to the desired concentration. Halothane was directly dissolved in Krebs' solution in an organ bath. Caffeine was initially dissolved in Krebs' solution to the desired concentration and then Krebs' solution was exchanged for caffeine-containing Krebs' solution in an organ bath.

2.3. Tissue Preparations. Rats were anesthetized with diethyl ether and were exsanguinated via the axillary arteries. The abdominal and thoracic cavities were opened, and the stomach, esophagus up to the larynx, heart, and lungs were removed and immediately placed in a dish containing precooled aerated Krebs' solution. A 5 mm long segment of the middle part of the thoracic esophagus was carefully dissected out for mechanical recording. The heart was also dissected out and retrogradely perfused through the aorta with Krebs' solution for washing out intracardiac blood. The right ventricle was longitudinally cut into strips of 1 cm in length, 2 mm in width, and 1 mm in thickness. For estimating mechanical activity of the representative skeletal muscle, hind limbs were exposed and the soleus was excised from both legs. The soleus muscle was also cut into strips of the same size as that of heart muscle strips. For connecting the heart and soleus muscle strips to the isometric tension transducer, a silk-snare was placed at an end of each strip.

2.4. Recordings of Mechanical Activity. The whole segment of each muscle preparation was transferred to a 3 mL thermostatically controlled (35°C) organ bath containing Krebs' solution bubbled with 95% O_2 + 5% CO_2 gas mixture, maintained at pH 7.4. For recording contractile responses of the esophageal segments, two L-shaped stainless-steel pins were introduced into the esophageal lumen; one pin was fixed to the bottom of the organ bath and the other was connected to the bar of the isometric force transducer (T7-30-240; Orientec, Tokyo, Japan). For recording contractile responses of the muscle strips isolated from the heart and the soleus muscle, one end was fixed to an L-shaped hook at the bath side and the other end was connected to an isometric tension transducer via a placed silk-snare. The contractile responses were recorded isometrically on PowerLab system (ADInstruments, Bella Vista NSW, Australia) through an AC amplifier (AS1202, NEC, Tokyo, Japan). An initial resting tension of 1 g was applied to each preparation, which was subsequently allowed to equilibrate for at least 30 minutes.

2.5. Electrical Field Stimulation. For inducing muscle contractile response, electrical field stimulation (EFS) was applied through a pair of platinum electrodes placed on either sides of each preparation. EFS was applied using an electronic stimulator (model SEN-3201, Nihon Kohden, Tokyo, Japan) connected to the electrodes. For EFS with single pulses, square-wave pulses of supramaximal intensity (80 V) and 10 ms in duration were applied at intervals of 2 minutes. For repeated multipulse EFS, square-wave pulses of supramaximal intensity (80 V) and 10 ms in duration were applied at frequency of 20 Hz for 1 s.

2.6. Data Processing and Statistical Analysis. Data are presented as means \pm standard deviation (S.D). Isometric contractile responses were given as g contraction/ mm^3 tissue volume. *n* indicates the number of separate preparations. The significance of differences between mean values was determined by one-way analysis of variance followed by

Tukey-Kramer's test. A P -value less than .05 denotes the presence of a statistically significant difference.

3. Results

3.1. EFS-Induced Contractile Responses of Esophageal, Skeletal, and Cardiac Muscles. To clarify the contractile properties, the mechanical responses to EFS were recorded in esophageal, skeletal, and cardiac muscle preparations. Spontaneous contractions occurred without electrical stimulations in some preparations of cardiac muscle but not in preparations of esophageal and skeletal muscles (data not shown). EFS with single pulses (voltage: 80 V, duration: 10 ms) evoked twitch-like contractions in all types of muscle preparations (Figure 1(a)). The contractile responses were abolished by tetrodotoxin ($1 \mu\text{M}$) in esophageal and skeletal muscles, whereas the blocker failed to affect the contractions in cardiac muscle (data not shown). The amplitude of the twitch-like contraction of esophageal muscle was larger than that of skeletal muscle and was comparable to that of cardiac muscle (Figure 1(a)). The duration of the twitch-like contraction of esophageal muscle was comparable to that of skeletal muscle but shorter than that of cardiac muscle (Figure 1(b)).

3.2. Characterization of Summation of Contraction in Esophageal, Skeletal, and Cardiac Muscles. To determine whether esophageal muscle shows summation of contractions, repeated EFS (80 V-10 ms, 20 Hz for 1 s) was applied to the preparations. Skeletal muscle and cardiac muscle preparations were also used to obtain positive and negative controls of summation, respectively. Repeated multipulse EFS evoked summation of contraction in skeletal muscle, resulting in tetanic contractions (Figure 2(b)). In contrast, cardiac muscle did not show summation of contraction (Figure 2(c)). Esophageal muscle preparation responded to all 20 individual stimulations and summated transiently in response to the initial several stimulations (Figure 2(a)). However, the response observed in esophageal muscle was different from that in skeletal muscle. Maximal amplitude of tetanic contractions was 2-3 times larger than that of twitch-like responses induced by single-pulse EFS in skeletal muscle (Figure 2(b)). Esophageal muscle reached a peak of tetanic contractions after 3-4 pulses, the amplitude of which was about 1.5 times larger than that of twitch-like responses (Figure 2(a)). Then an amplitude similar to that of the twitch-like contraction was sustained until the end of the stimulations (Figure 2(a)).

3.3. Effects of an Inhibitor of Gap Junctions on EFS-Induced Contractions in Esophageal and Cardiac Muscles. To clarify the involvement of gap junctions in the contractile responses, we examined the effects of an inhibitor of gap junctions, halothane, on the EFS-induced contractions in esophageal and cardiac muscles. Application of halothane (50 mM) inhibited the contractions in cardiac muscle (Figure 3(b)). In

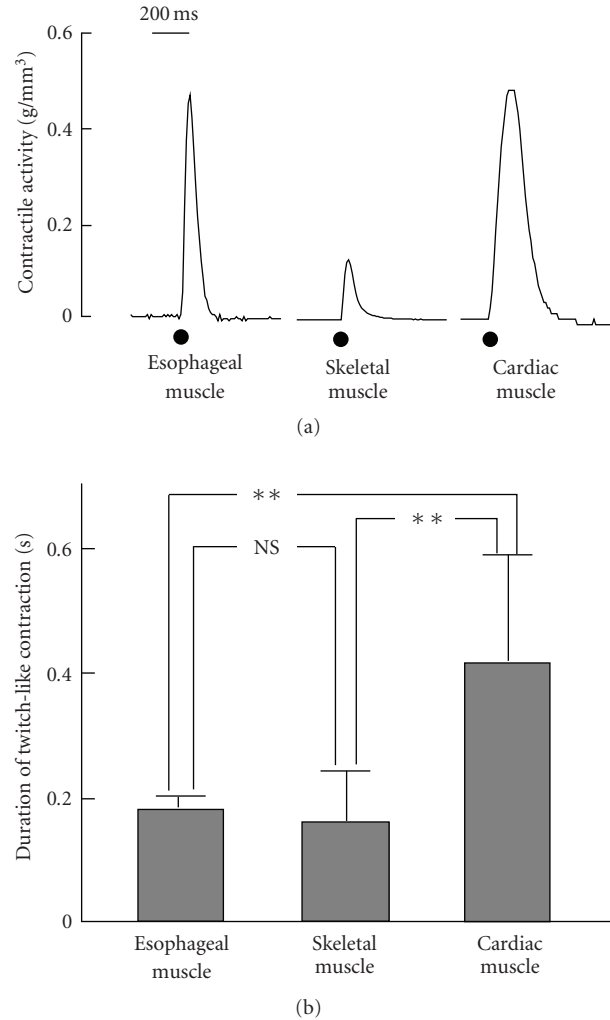


FIGURE 1: Single-pulse electrical stimulation-induced twitch-like contractions of esophageal, skeletal, and cardiac muscles in rats. (a) Representative traces of the contractile responses to electrical field stimulations (EFS) in esophageal, skeletal, and cardiac muscle preparations are shown. EFS was applied by using single pulses (voltage: 80 V, duration: 10 ms). Closed circles indicate the points of EFS. (b) Summary graphs of the duration of the EFS-evoked twitch-like contractions in esophageal ($n = 16$), skeletal ($n = 6$), and cardiac ($n = 5$) muscle preparations. Each bar represents the mean \pm S.D. ** shows significant difference ($P < .01$). NS shows no significant difference.

contrast, esophageal muscle contractions were not affected by halothane (Figure 3(a)).

3.4. Contractile Responses Induced by High Potassium Stimulation in Esophageal, Skeletal, and Cardiac Muscles. We then compared the effects of high potassium stimulation on the contractile responses in the three muscles. Application of high potassium (118.4 mM) in the organ bath evoked phasic contractions in esophageal and skeletal muscles (Figure 4(a)). In contrast, cardiac muscle showed long-lasting contractions, which were sustained at least for 2 minutes.

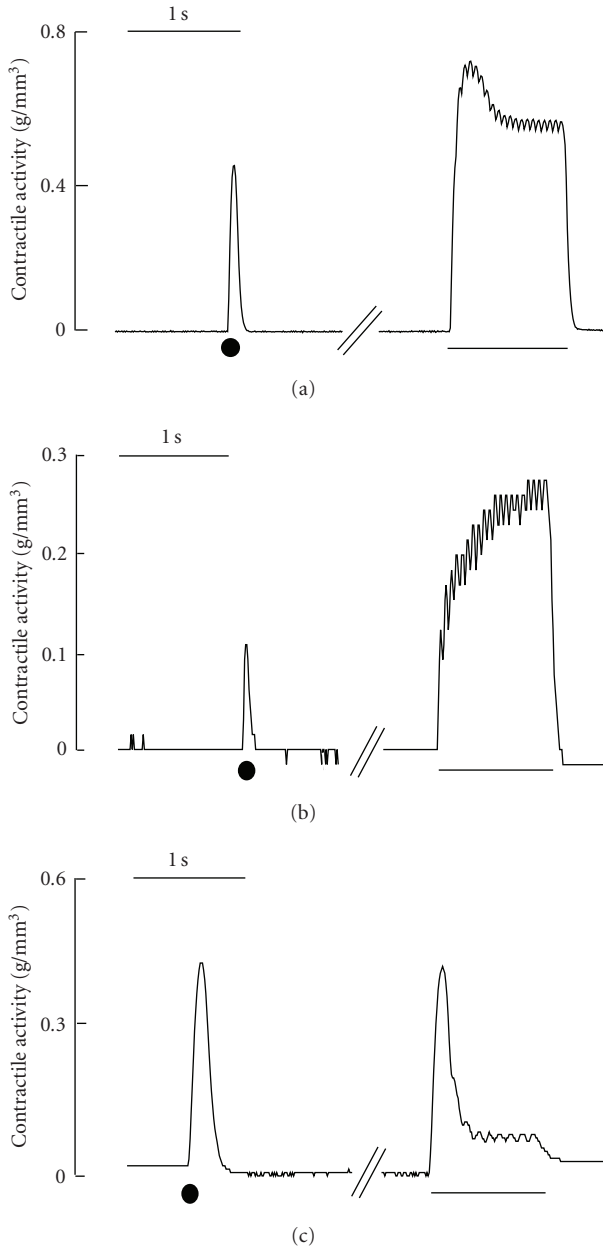


FIGURE 2: Multipulse electrical stimulation-induced contractile responses of esophageal, skeletal, and cardiac muscles. Representative traces of the contractile responses to single and multipulse repeated EFS in esophageal (a), skeletal (b), and cardiac (c) muscle preparations are shown. Similar results were reproducibly obtained in five independent experiments. EFS was applied by using single or repeated pulses (80 V-10 ms, 20 Hz for 1 s). Closed circles and underbars indicate the points of single EFS and the location of multiple EFS, respectively.

3.5. Contractile Responses Induced by Caffeine in Esophageal, Skeletal, and Cardiac Muscles. Exogenous application of caffeine (20 mM), a releaser of calcium from intracellular stores, caused two-phase contractions in esophageal and skeletal muscles (Figure 4(b)). First-phase contraction occurred just after application of caffeine and sustained the tension for

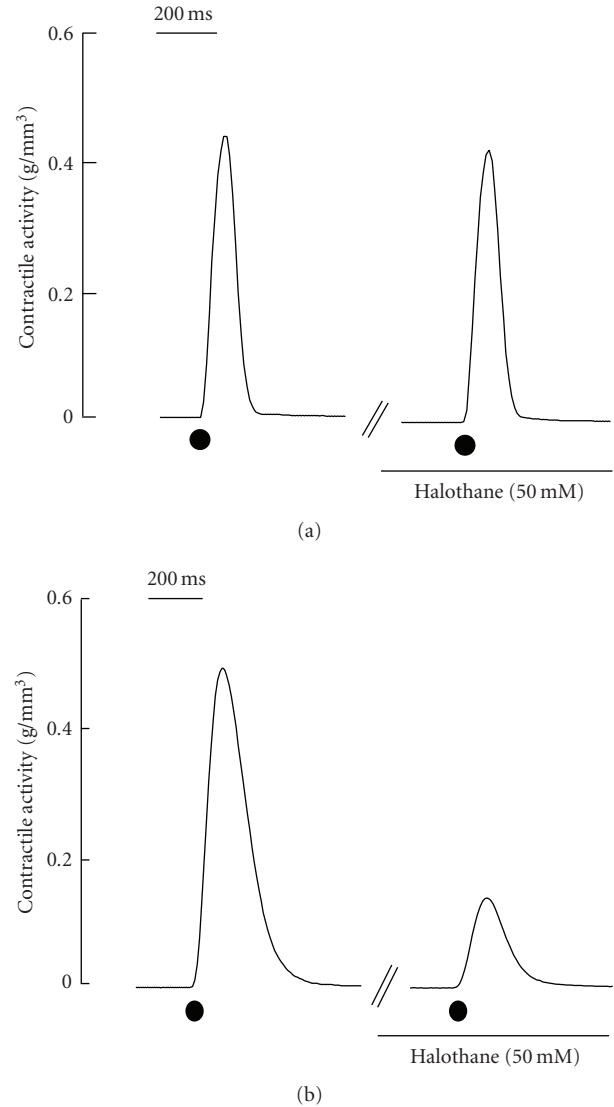


FIGURE 3: Effects of halothane on the contractile responses in esophageal and cardiac muscles. Single-pulse EFS was applied in the absence and presence of halothane (50 mM), an inhibitor of gap junctions, and the contractile responses were recorded. Representative traces of esophageal (a) and cardiac (b) muscle preparations are shown. Similar results were reproducibly obtained in four independent experiments.

about 5 minutes. After the first contraction, the tension was increased again, inducing the second-phase contraction (Figure 4(b)). On the other hand, cardiac muscle showed a small sustained contraction.

4. Discussion

In the present study, we investigated the contractile properties of esophageal striated muscle by comparison with those of other types of striated muscle, that is, skeletal and cardiac muscles. The major findings are (1) EFS with single pulses evoked twitch-like contractile responses in esophageal muscle, similar to those in skeletal muscle in duration and

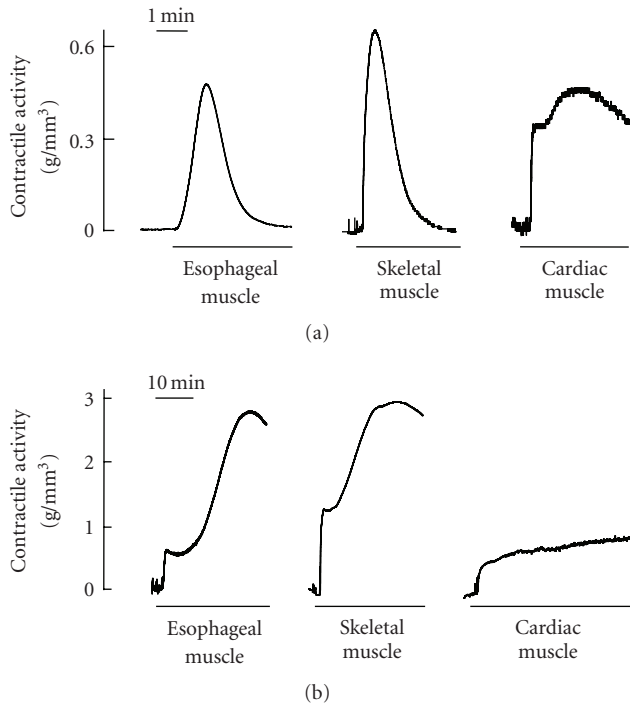


FIGURE 4: Contractile responses induced by high potassium or caffeine in esophageal, skeletal, and cardiac muscles. Muscle preparations were stimulated with high potassium (a) or caffeine (b). Representative traces of the contractile responses in esophageal, skeletal and cardiac preparations are shown. Similar results were reproducibly obtained in five independent experiments. Underbars indicate the application of high potassium (118.4 mM) and caffeine (20 mM).

similar to those in cardiac muscle in amplitude, (2) EFS with a high frequency induced a transient summation of contractions followed by a sustained contraction with an amplitude comparable to that of twitch-like contraction induced by the single-pulse EFS, (3) an inhibitor of gap junctions did not affect the esophageal contractions, and (4) both high potassium stimulation and application of caffeine induced contractions of esophageal muscle in a manner analogous to that of skeletal muscle. These findings suggest that contractile properties of esophageal striated muscle are not identical to those of skeletal muscle. The specific characteristics revealed in the present study might be associated with effective peristalsis in the striated muscle portion of the esophagus.

It has generally been accepted that striated muscle fibers in the esophagus are a type of “skeletal” muscle [3, 5, 6]. In agreement with this, esophageal striated muscle showed properties similar to those of skeletal muscle (soleus muscle) in terms of duration of twitch-like contraction, sensitivity to tetrodotoxin, and responses to high potassium or caffeine. It was of interest to determine whether esophageal striated muscle has the ability to contract spontaneously, but it never showed contractile activity unless appropriate stimulus was applied. This is also in line with the general concept that esophageal muscle fibers are skeletal muscles. However,

summation of the contractions during tetanic stimulation was not obvious in the esophageal muscle, although large summation of contractions is one of the important characteristics of skeletal muscles. Moreover, large amplitude of twitch-like contraction in the esophageal muscle after application of a single-pulse stimulus is analogous to that in cardiac, but not skeletal, muscle. It is therefore thought that esophageal striated muscle is similar but not identical to skeletal muscle.

Low amplitude in twitch-like contraction is a potential basis for large summation of contractions in skeletal muscle since it provides a wide margin to summate sequential contractions. It is thus reasonable to expect that the modest summation of contractions in the esophageal muscle may be related, at least in part, to the large amplitude of contraction in response to single-pulse stimulation. In the case of cardiac muscle, the large amplitude of twitch-like contraction can contribute to generation of sufficient ventricular pressure during isovolumic contraction and to effective reduction of ventricular end-systolic volume [28–30]. The absence of summation is also essential to ensure diastolic intervals for ventricular filling [30]. Considering that properties of cardiac muscle are adequately utilized to accomplish effective pumping out of blood in the heart, the properties of esophageal muscle would be suitable for the esophageal peristalsis. In contrast to small and large intestines, where intraluminal contents are usually chyme, the esophagus needs to propel solid foods rapidly. For this purpose, rapid and large contraction of the esophageal muscle in response to neural messages may be convenient. Furthermore, esophageal function is specialized for propulsion, but not agitation or retention [7, 16]. It is therefore most probable that keeping the maximal contraction at a particular portion of the esophagus is dispensable but that dilation of muscle after propelling the intraluminal contents is required to receive additional foods or to prevent stagnation. Accordingly, it seems likely that only a transient summation of contractions, which is one of the specific properties of esophageal muscle, is suitable for the function of the esophagus.

In the cardiac muscle preparation, EFS applied at 20 Hz caused one complete twitch-like contraction followed by small rise in tension. It is well established that the lack of summation in cardiac muscle depends on a long-lasting refractory period of the action potential, which results in substantial overlapping between the refractory period and duration of contraction [30]. However, the refractory period of esophageal muscle would not overlap with its contraction. This is based on the fact that esophageal muscle contractions induced by high-frequency stimulation were maintained after the transient summation, with the amplitudes being comparable to those of twitch-like contractions. Taking into consideration the fact that strength of striated muscle contraction is correlated with intracellular calcium concentration [31], it can be postulated that esophageal muscle has the ability to keep calcium level at a constant level during repeated stimulation. Since caffeine, a calcium releaser from the intracellular calcium store, caused similar tetanic contractions in esophageal and skeletal muscles, it is

possible that calcium uptake into the store is unique in the esophageal striated muscle.

Electrical coupling of cardiac muscle cells through gap junctions is essential for coordinated contraction [32, 33]. In the present study, we tried to elucidate the possible involvement of gap junctions in coordination of the contractions of esophageal striated muscles. An inhibitor of gap junctions, halothane, failed to affect EFS-induced contractile responses in esophageal muscle, whereas the inhibitor blocked those in cardiac muscle. Gap junctional protein connexin 43, which is a major connexin expressed in cardiac muscles [32], was not detected in esophageal muscles in our immunohistochemical analysis (unpublished observation). At present, the most probable conclusion is that esophageal muscles do not couple to each other through gap junction channels. However, gap junction channels are formed by a family of more than 20 connexin proteins [34, 35], and halothane might not inhibit all of the gap junction proteins. Hence, there remains the possibility that the halothane-insensitive channels are operated in the esophageal muscles.

5. Conclusion

We characterized the contractile properties of esophageal muscle in comparison with those of skeletal and cardiac muscles in rats. The results demonstrate that esophageal muscle has properties that are similar but not identical to those of skeletal muscle. It is thought that some specific properties of esophageal muscle may be beneficial for peristaltic motility in the esophagus.

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