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Masafumi Fujii* Tatsuji Namba†

*Okayama University,

†Okayama University,

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Abstract

The cholinesterase activity of skeletal muscle and its subcellular components, including motor endplates, was compared chemically in human, mouse and rat. The total cholinesterase activity of muscle per unit protein was in the descending order of human, mouse and rat. Cholinesterase was present in all subcellular components fractionated by differential centrifugation, and was greatest in the microsome fraction followed, in descending order, by the mitochondria, myofibril, and supernatant fractions. Each of these fractions had greater cholinesterase activity in human muscle than in mouse muscle, and in mouse muscle than in rat muscle. The ratio of the activity of the microsome fraction to the activity of muscle homogenate was 11.1 in human, 4.6 in mouse and 3.4 in rat. Because of its relatively greater proportion, the myofibril fraction seems to contribute most to the total cholinesterase activity of muscle. Muscle membrane contained high cholinesterase activity of motor endplates, and the activity was greater than the activity of the microsome fraction in rat. Cholinesterase activity per motor endplate was in the descending order of rat, human and mouse, and the variation was less than the variation in the total muscle cholinesterase activity among these species.

KEYWORDS: choinesterase, skeletal muscle, subcellular componenets, muscle membrane, motor endplate

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CHOLINESTERASE OF SKELETAL MUSCLE AND ITS SUBCELLULAR COMPONENTS

Masafumi FUJII and Tatsuji NAMBA

Second Department of Internal Medicine, Okayama University Medical School, Okayama 700, Japan (Director: Prof. I. Kimura) and Department of Medicine, Maimonides Medical Center, and State University of New York, Downstate Medical Center, College of Medicine, Brooklyn, New York 11219, U.S.A.

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Abstract. The cholinesterase activity of skeletal muscle and its subcellular components, including motor endplates, was compared chemically in human, mouse and rat. The total cholinesterase activity of muscle per unit protein was in the descending order of human, mouse and rat. Cholinesterase was present in all subcellular components fractionated by differential centrifugation, and was greatest in the microsome fraction followed, in descending order, by the mitochondria, myofibril, and supernatant fractions. Each of these fractions had greater cholinesterase activity in human muscle than in mouse muscle, and in mouse muscle than in rat muscle. The ratio of the activity of the microsome fraction to the activity of muscle homogenate was 11.1 in human, 4.6 in mouse and 3.4 in rat. Because of its relatively greater proportion, the myofibril fraction seems to contribute most to the total cholinesterase activity of muscle. Muscle membrane contained high cholinesterase activity of motor endplates, and the activity was greater than the activity of the microsome fraction in rat. Cholinesterase activity per motor endplate was in the descending order of rat, human and mouse, and the variation was less than the variation in the total muscle cholinesterase activity among these species.

Key words: cholinesterase, skeletal muscle, subcellular components, muscle membrane, motor endplate.

Cholinesterase (ChE) plays a key role, together with acetylcholine and acetylcholine receptor, in cholinergic transmission including neuromuscular transmission. ChE of the neuromuscular junction may play a role in the pathophysiology of diseases of neuromuscular transmission, and in the action of drugs which affect neuromuscular transmission. The function of non-endplate ChE is not known, but probably plays a very minor role, if any, in neuromuscular transmission. This paper describes comparative studies on ChE activity of skeletal muscle and its subcellular components, including motor endplates, of human, mouse and rat.

MATERIALS AND METHODS

Human intercostal muscle was obtained during postmortem examination within several

hours of death from patients without neuromuscular diseases. Skeletal muscles of Swiss mice and Wistar rats were obtained immediately after death by pentobarbital sodium anesthesia. Mice and rats had mixed muscles. The muscle was homogenized in a Polytron homogenizer with phosphate-buffered saline solution containing 150 mM NaCl-20 mM sodium phosphate buffer, pH 7.4. The homogenate was centrifuged at 400 g for 3 min, and the supernatant fraction was successively centrifuged. The following fractions were precipitated: at 600 g for 3 min for the myofibril fraction; 13,500 g for 25 min for the mitochondria fraction; and 115,000 g for 90 min for the microsome fraction. The remaining supernatant fluid was recovered as the supernatant fraction. Each particulate fraction was purified by repeated suspension in phosphate-buffered saline solution and centrifugation. The purity of each fraction was determined under a phase-contrast microscope. Muscle membrane was prepared from homogenate of intercostal muscle by the method of Namba and Grob (1) with a slight modification. The muscle was homogenized in a Polytron homogenizer with 50 mM $CaCl_2$ solution, and filtered through an 18 mesh plastic net. The filtrate was centrifuged at 1,000 g for 1 min, and the precipitate was washed three times with buffered KCl solution, containing 45 mM KCl, 30 mM $KHCO_3$, 2.5 mM dl-histidine monohydrochloride, adjusted to pH 7.8 with 1 M tris hydroxymethylaminomethane solution. The precipitate was suspended in buffered KCl solution, incubated in a water bath at 37 ℃ for 30 min, and left at 4 ℃ for 20 min. The suspension was centrifuged at 1,000 g for 1 min, and the precipitate was washed twice with 2.5×10^{-7} M NaOH solution and then left overnight suspended in the solution at 4 ℃. The suspension was washed twice with 25 mM glycine-NaOH buffer, pH 10.7. The membrane preparation was obtained after washing the suspension twice with 2.5×10^{-7} M NaOH solution.

ChE activity was determined by the hydroxamic acid method (2). Incubation was performed at 37 ℃ for 30 min in a medium containing 4 mM acetylcholine bromide, 100 mM NaCl, 10 mM $MgCl_2$ and 30 mM sodium phosphate buffer, pH 7.5. Protein was measured by the method of Lowry *et al.* (3) with bovine serum albumin as the standard.

The ChE activity of the motor endplate was determined by dividing the ChE activity of an aliquot of muscle membrane preparation by the number of motor endplates in the aliquot since ChE was found solely in the motor endplates in this preparation (1, 4).

RESULTS

ChE activity of muscle fibers was determined in the homogenate of skeletal muscle, which had activity per unit protein in the descending order of human ($p < 0.001$), mouse ($p < 0.001$) and rat (Table 1). The ChE activity of human

TABLE 1. CHOLINESTERASE ACTIVITY OF SKELETAL MUSCLE[a]

		Supernatant			
	Homogenate	Myofibrils	Mitochondria	Microsomes	fluid
Human	440 ± 48	438 ± 23	1,202 ± 46	4,903 ± 136	294 ± 26
Mouse	132 ± 6	176 ± 12	242 ± 17	602 ± 21	112 ± 5
Rat	53 ± 4	61 ± 4	89 ± 9	180 ± 13	42 ± 5

a, Nanomoles of acetylcholine hydrolyzed/30 min/mg protein; mean ± S.E.M. in 15 samples.

muscle was 8.3 times the activity of mouse muscle.

ChE activity of subcellular components of skeletal muscle per unit protein was greatest in the microsome fraction ($p < 0.001$), which was followed, in descending order, by the mitochondria, myofibril and supernatant fractions. The activity per unit protein of the microsome fraction compared with the activity of the homogenate was 11.1 times in human muscle, 4.6 times in mouse muscle, and 3.4 times in rat muscle. The activity of each fraction was greater in human muscle than in mouse muscle ($p < 0.001$), and was greater in mouse muscle than in rat muscle ($p < 0.001$).

The ChE activity of motor endplates in the intercostal muscle was in the descending order of rat ($p < 0.05$), human and mouse (Table 2). The mean ChE activity of a rat motor endplate was 3.7 times that of a mouse motor endplate. The muscle membrane of the intercostal muscle had a ChE activity per unit protein in the descending order of rat ($p < 0.01$), human ($p < 0.001$) and mouse. The ratio of ChE activity per unit of muscle membrane to the activity of muscle homogenate was in inverse proportion to the activity of muscle homogenate, since the range of the ChE activity among species was smaller in the muscle membrane than in the muscle homogenate. The ChE activity of the membrane fraction was greater than other fractions only in the rat.

TABLE 2. CHOLINESTERASE ACTIVITY OF MOTOR ENDPLATE AND MUSCLE MEMBRANE IN INTERCOSTAL MUSCLE

	Cholinesterase activity[a]	
	Per motor endplate	Per mg protein
Human	0.40 ± 0.11	1,251 ± 86
Mouse	0.24 ± 0.06	572 ± 42
Rat	0.76 ± 0.10	1,628 ± 73

a, Nanomoles of acetylcholine hydrolyzed/30 min; mean ± S.E.M. in 15 samples.

DISCUSSION

Histochemical and biochemical localization reveal that ChE is largely concentrated at the motor endplates of skeletal muscle (1, 4). The function of ChE at the motor endplate is considered to be termination of neuromuscular transmission via the hydrolytic inactivation of acetylcholine. The presence of ChE in other constituents of skeletal muscle has not been well appreciated, although motor endplate ChE accounts for less than 20% of the total muscle ChE (4).

ChE in myofibrils seems to be mainly myosin ChE, which is localized in light meromyosin (5, 6). The myofibril fraction probably contributes most to the total ChE activity of muscle since the relative quantity of myofibrils is greatest among subcellular components of skeletal muscle. Electron microscopically,

ChE activity has beeh demonstrated at the lateral part of the A-band in rat rectus superior muscle (7).

ChE activity has also been demonstrated electron microscopically in mitochondria of skeletal muscle (8, 9) and of motor nerve endings (9).

High ChE activity in microsomes was found in muscles of three species in the present study and is in accord with the report on human muscle (10). Electron microscopically, ChE activity was localized in both the transverse tubule and sarcoplasmic reticulum in frog sartorius muscle (8). However, ChE activity was demonstrated in the transverse tubule, but not in the sarcoplasmic reticulum, in rabbit psoas muscle (11) and rat rectus superior muscle (7). ChE in the sarcotubular system may play a role in propagation of impulses within the muscle fiber, but there is only indirect evidence indicating such a role. Tetanic contractions of skeletal muscle are accompanied by a rise in ChE activity in muscle sarcoplasmic reticulm (12). Sodium transport in frog sartorius muscle was inhibited by greater concentrations of ChE inhibitor (13). After denervation at birth, ChE active spots developed in the regions of rat muscle fibers lacking motor endplates (14). Although morphological identification has not been made, these spots may have developed from the sarcotubular system.

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