*Acta Medica Okayama*

*Volume* 36, *Issue* 3 1982 *Article* 9

JUNE 1982

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## Abstract

Cholinesterase activity was localized solely in the motor endplate of the membrane in rate intercostal muscle. The diameter of rat motor endplates in the gradient dimension was 31.9 micrometers. The cholinesterase activity per unit protein of the soluble fraction of rat muscle membrane was 35.6% higher than the original membrane. From studies with specific substrates and cholinesterase inhibitors, the cholinesterase activity of rat muscle membrane and its soluble fraction consists of more than 90% acetylcholinesterase and less than 10% pseudocholinesterase.

**KEYWORDS:** cholinesterase, acetycholinesterase, pseudocholinesterase, rat motor endplate, cholinesterase inhibitor

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*PMID: 7113749 [PubMed - indexed for MEDLINE]

Acta Med. Okayama 36, (3), 233–236 (1982)

## —— BRIEF NOTE ——

# CHOLINESTERASE ACTIVITY OF THE MOTOR ENDPLATE IN RAT INTERCOSTAL MUSCLE

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*Received November 6, 1981*

*Abstract.* Cholinesterase activity was localized solely in the motor endplate of the membrane in rat intercostal muscle. The diameter of rat motor endplates in the greatest dimension was 31.9 $\mu$m. The cholinesterase activity per unit protein of the soluble fraction of rat muscle membrane was 35.6% higher than the original membrane. From studies with specific substrates and cholinesterase inhibitors, the cholinesterase activity of rat muscle membrane and its soluble fraction consists of more than 90% acetylcholinesterase and less than 10% pseudocholinesterase.

*Key words :* cholinesterase, acetylcholinesterase, pseudocholinesterase, rat motor endplate, cholinesterase inhibitor.

Cholinesterase (ChE) is present at motor endplates and its hydrolysis of acetylcholine plays an important role in regulating muscle contraction. We previously found that the ChE activity of rat muscle homogenate was only 21.4% of the activity of human muscle homogenate (1). In the present study, the activity and properties of ChE of the motor endplate in rat skeletal muscle were studied in isolated muscle membrane.

Muscle membrane was prepared from a homogenate of the intercostal muscle of Wistar rat by removing intracellular components as described previously (2). The ChE activity was determined by the hydroxamic acid method (3), incubating samples for 30 min at 37 ℃ 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.* (4). Staining for ChE activity of the membrane was performed by the method of Karnovsky and Roots (5). The diameter of motor endplates was measured in teased muscle fibers with an ocular micrometer.

Numerous rat motor endplates were visualized by staining isolated membranes for ChE on a Millipore filter (Fig. 1). The fine structures of these endplates were similar to structures of endplates demonstrated in muscle sections stained for ChE. The diameter of rat motor endplates in the greatest dimen-

sion was 31.9 ± 0.79 $\mu$m (mean ± SEM in 500 motor endplates) (Fig. 2). In 15 rat muscle membranes 37.9 ± 0.46% of the total motor endplate ChE was solubilized by 0.5% Triton X-100. As calculated per mg protein in each fraction, ChE activity was 1.63 ± 0.034 ($\mu$moles acetylcholine hydrolyzed in 30 min per mg protein) in 15 muscle membranes, 2.21 ± 0.047 in 15 soluble fractions and 1.14 ± 0.034 in 15 insoluble fractions. The mean specific activity of the

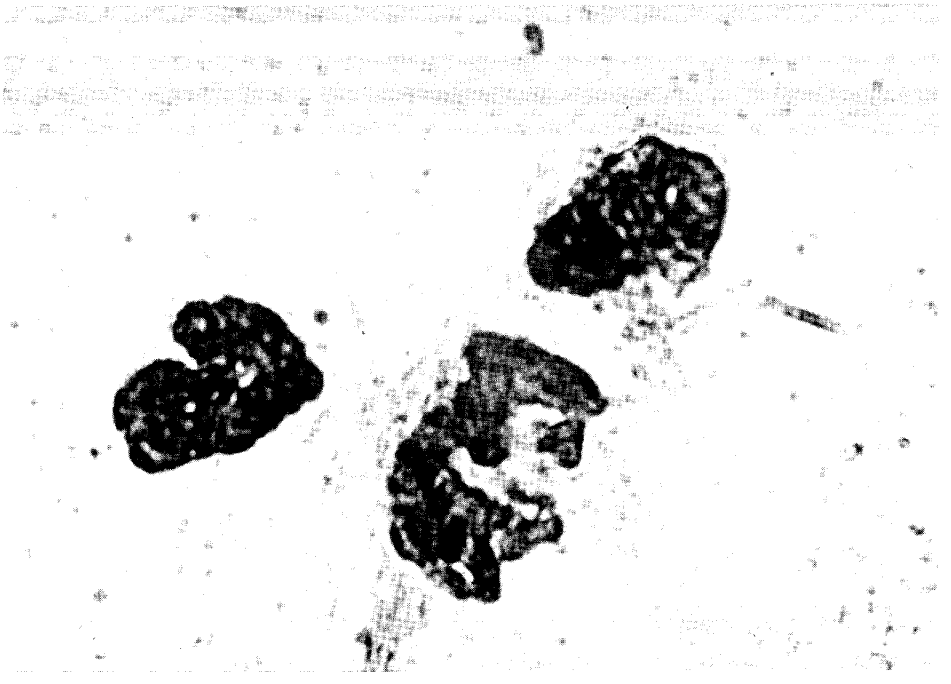

Fig. 1. Rat motor endplates in the membrane preparation, demonstrated by cholinesterase staining. × 1642.

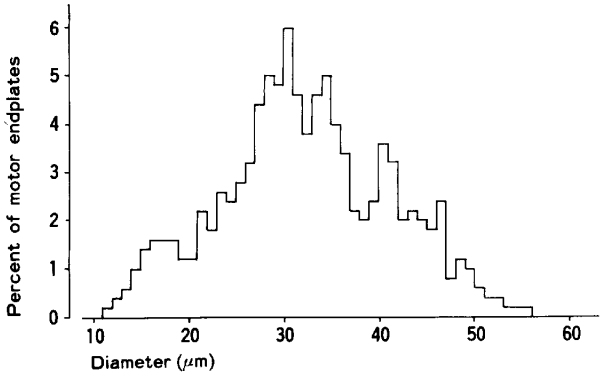


Fig. 2. The maximum diameter of the motor endplates in rat intercostal muscle.

soluble fraction was 35.6% higher than the muscle membrane. Using acetyl-beta-methylcholine as substrate, the ChE activity was 1.30 ± 0.074 in 8 membranes, 1.88 ± 0.139 in 8 soluble fractions and 1.10 ± 0.137 in 8 insoluble fractions. Using butyrylcholine as substrate, the ChE activity was 0.150 ± 0.0197 in 8 membranes, 0.006 ± 0.0061 in 8 soluble fractions and 0.025 ± 0.0071 in 8 insoluble fractions. The types of ChE activity in rat muscle membrane and its soluble fraction were determined by the effect of inhibitors of pseudocholinesterase and of acetylcholinesterase. Diisopropylfluorophosphate, which inhibits 100% of pseudocholinesterase at concentrations of $10^{-8}$ M or higher, and less than 5% of acetylcholinesterase at $10^{-7}$ M, inhibited the ChE activity of the muscle membrane by 9.9% at $10^{-7}$ M, and of soluble fraction by 5.9% at $10^{-7}$ M. 1, 5-bis-(4-allyldimethylammoniumphenyl) pentan-3-one dibromide (BW284C51), which inhibits more than 95% of acetylcholinesterase and less than 5% of pseudocholinesterase at concentrations between $10^{-6}$ and $10^{-5}$ M, completely inhibited ChE activity of the soluble fraction at $10^{-6}$ M, while 8.8% of ChE activity of muscle membrane remained in the presence of $10^{-6}$ M BW284C51. ChE activity of rat muscle membrane and its soluble fraction therefore consists of more than 90% acetylcholinesterase and less than 10% pseudocholinesterase. The amount of acetylcholine hydrolized by rat muscle membrane and its soluble fraction increased with increasing concentration of acetylcholine between 0.5 and 8.0 mM (Fig. 3). When the results were plotted

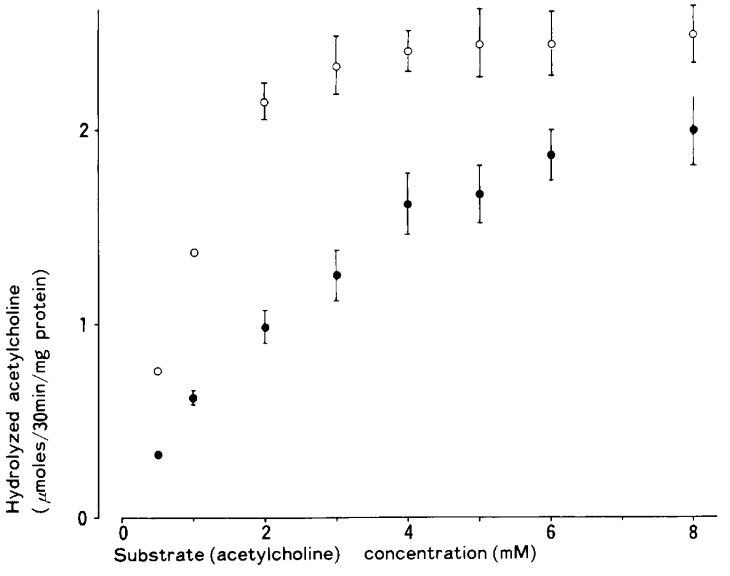


Fig. 3. Effect of substrate concentration on cholinesterase activity of the isolated rat muscle membrane (●——●) and its soluble fraction (○——○). At each substrate concentration, 24 estimations in muscle membrane and its soluble fraction were performed. Vertical lines indicate the standard error of the mean.

according to the method of Woolf (6) (Fig. 4), the Michaelis-Menten constant (Km) was 3.39 mM in muscle membrane and 0.60 mM in soluble fraction.

The isolated skeletal muscle membrane enabled determination of the ChE activity of rat motor endplate. The preparation should also be useful for the study of other properties of rat motor endplates.

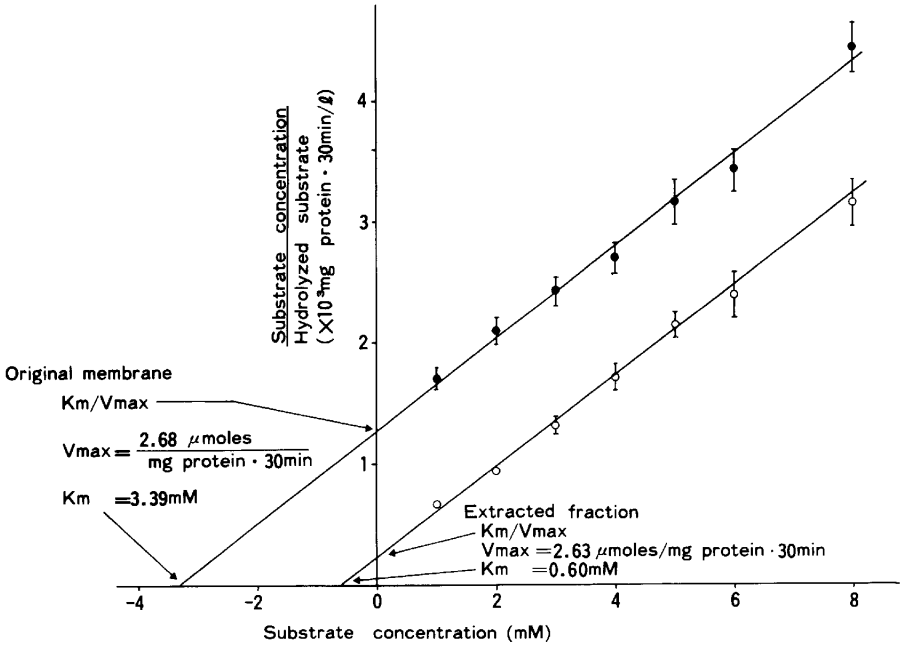


Fig. 4. The same data as in Fig. 3 plotted by the method of Woolf.

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