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Scanning electron microscopic image of neuromuscular junctions

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Introduction

Although recent electron microscopical studies have added much information to the structure and function of neuromuscular junctions(NMJs), there is very little information in literature concerning three dimensional organization of NMJs at the fine structural level.

So far scanning electron microscopic studies of NMJs have not been achieved; the main obstacle resides in the presence of intramuscular connective tissue components which totally conceal the surface of NMJs when examined under the scanning microscope.

To overcome this problem, we have applied a modification of the HCl-Collagenase method which has been introduced by Evan *et al.*[1] in the survey of the basal aspect of tissues and cells.

Materials and method

The sternothyroid muscle of the chinese hamster were used for this study. The muscles were fixed *in situ* buffered glutaraldehyde followed by postosmification. In order to remove connective tissue components, the muscle strips were treated with 8N HCl for 20 to 40 min at 60°C after rinsing them in distilled water according to the method reported by Evan *et al.*[1] It was found that the treatment with HCl alone removes collagen and basal lamina almost completely, and that the successive digestion with collagenase recommended in the original method was found to be omissible. Another method for the same purpose which consists of enzymatic digestion prior to fixation[2] was also tested but resulted in serious distortion of the tissues caused by muscle contraction during the specimen preparation. They were dehydrated through a graded series of ethanol and were immersed in isoamyl acetate for 30 min. After drying by the critical point method and spatter coating with gold, the specimens were examined in Hitachi S-500A scanning electron microscope.

Results and discussion

In the specimens where intramuscular connective tissues are adequately removed, such structures as muscle fibres, intramuscular nerves and blood capillaries are immediately recognized under the scanning microscopy.

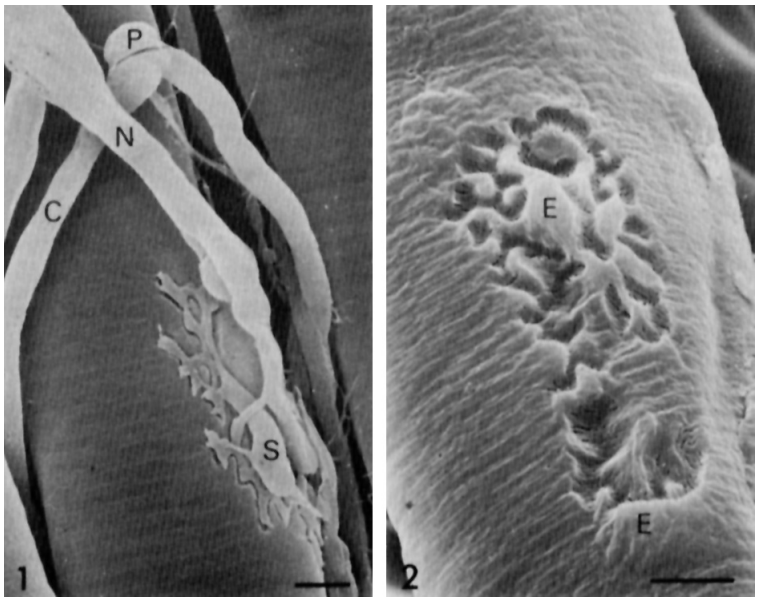

Fig. 1. Scanning electron microscopic image of NMJ of the chinese hamster. A branch of motor nerves (N) tapers to form a ramifying nerve ending which is fitted into subsynaptic depression of the muscle surface. (S); Schwann cell body. (C); Capillary with a pericyte (P).
Bar indicates 5 μm

Fig. 2. En face view of subsynaptic depression, showing the shape and arrangement of the junctional folds. Sarcoplasmic eminences (E) are evident. In this preparation, the nerve ending has been detached from the muscle surface during the HCl hydrolysis.
Bar indicates 5 μm

The nerves are found to ramify repeatedly in the vicinity of NMJs, and each branch tapers abruptly to give rise to a nerve ending; the branching point apparently represents the last node of Ranvier. The nerve endings are closely applied to the muscle surface lying in a set of depressions of muscle fibre surface to form a NMJs. The NMJs cover an oval area of about 15 by 30 μm in which bulged Schwann cell bodies are evident. A number of thin ramifying nerve endings are spread out of the cell bodies, which often appear to overlap and join each other to form a rather complicated texture (Fig. 1).

The surface of the nerve endings was exposed with this method but terminal axons themselves were not visualized owing to the presence of the Schwann cells covering. However, it is known from the previous transmission electron microscopic study that the Schwann cell covering is generally extremly attenuated, and therefore, the overall profile of the nerve endings is thought to conform closely to the shapes of the terminal axon, except in the region of the nuclei.

In addition to the surface features, the subsynaptic organization of the sarcolemma is visualized in those specimens in which the nerve endings have been detached from the muscle surface during the specimen preparation, possibly caused by hydrolysing materials of basal lamina in the synaptic clefts. In these specimens, the junctional sarcolemma exhibits deep and irregular synaptic depressions which are incompletely partitioned by ridges or folds of the junctional sarcoplasm. Unlike the schematic drawing of Couteaux[3] the junc-

tional folds are randomly disposed with respect to the long axis of the muscle fibre. The complexity in the shape and arrangement of these folds is fully displayed in this type of preparation (Fig. 2). Elevations of the muscle fibre which show a smooth surface and lack any cross striation are seen in and around the synaptic depression. The elevations undoubtedly correspond to the "terminal cone or eminence" of Doyére,[4] which are known to contain muscle (or fundamental) nuclei, an accumulation of mitochondria and sarcoplasmic reticulum.

The present study extends the previous light and transmission electron microscopical findings, adding new information to the morphology of NMJs which may allow quantitative study of the postsynaptic organization of the NMJs. Scanning microscopic study of muscle spindles is also in progress in our laboratory.

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High potassium effect on the mobility of sea urchin sperm

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Immobilization of sea urchin sperm by high concentration potassium cation was first described by Gray[1] and has been examined by various authors.[2,3] It would have been selected as a typical example for electrophysiological study of flagellum motility if the sperm was not so small. Recently, new techniques for measurement of membrane potential and intracellular pH were developed for such small cells using lipid soluble radioactive cation[4] and ^{31}P Nuclear Magnetic Resonance spectrometer.[5] In this report the membrane potential and intracellular pH of sea urchin sperm are described in connection with the sperm motility under high potassium concentration.

Sea urchin, *pseudocentrotus depressus,* was obtained from Misaki Marine Biological Station and its sperm was shed by conventional method. The preparations were made by dilution of the sperm into the artificial sea water of the various concentration of potassium with 10 % of volume concentration.

Tritiated TPP (Tri-phenyl-methyl-phosphonium) cation was purchased from New England Nuclear and the ethanol containing original solution was diluted 1000 times when it was used. The membrane potential was calculated from the following equations,

$$= 2.3 \frac{RT}{F} \log \frac{C_{sol}}{C_{cell}} \qquad (1)$$