DENERVATION OF SKELETAL MUSCLE: CHANGES IN THE STRUCTURE OF NON-SYNAPTIC SARCOLEMMA

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1. Introduction

It is well known that in adult skeletal muscle acetylcholine (Ach) receptors are localized in the subsynaptic sarcolemma [4,5]. Denervation of muscle results in appearance of Ach receptors over the entire surface of the sarcolemma [6,7]. This increase of Ach receptors is due to incorporation of newly synthesized receptor proteins into the non-synaptic sarcolemma [2]. The biochemical and electron microscopic studies on receptor rich membranes isolated from electric organ of fish such as *Electrophorus electricus* and *Torpedo marmorata* indicate that the receptor protein spans the entire thickness of the membrane and is exposed at its surface [3]. Therefore, it is of interest to investigate the structural aspects of denervated sarcolemma at a stage when extra-junctional Ach sensitivity is known to be high [6], by using freeze-fracturing technique which enables a direct visualization of the interior of membranes [1].

This paper deals with our observations on the freeze-fractured sarcolemma from denervated skeletal muscle.

2. Material and methods

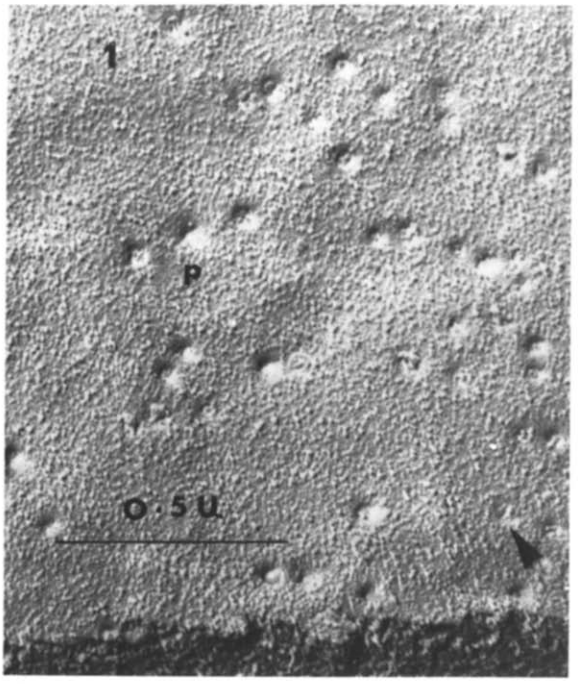
Lumbrical muscles of the left hind leg of the female rats weighing 120–150 g were denervated by sectioning the sciatic nerve in the upper thigh region. The muscles of the right leg served as controls. The animals were sacrificed at 2 weeks after denervation and the excised muscles were fixed in 2% glutaraldehyde in 0.1 M Cacodylate buffer (pH 7.2). The fixation was carried at 4°C for 2 h. After a brief rinse in cacodylate buffer, the muscles were soaked in 30% glycerol for 2 h. Small pieces of the muscles were then frozen in liquid Freon 22. Pt-c replicas were made after fracturing at −100°C in a Balzers BA 360 M high vaccum freeze-etch unit. The replicas were cleaned overnight in chromic acid and examined in Philips 300 electron microscope at an accelerating voltage of 60 or 80 kV. The original pictures were magnified to X 80 000 for measurement of particles. The results reported in this paper are based on a study of at least 12 replicas prepared from 12 muscles taken out of 3 denervated rats and many more replicas of normal muscles taken out from at least 10 animals.

3. Results

The nomenclature used by Branton et al. [1] for labelling fractured faces of biological membranes has been adapted in this paper. Therefore the fractured face of the sarcolemma on the cytoplasmic side as viewed from outside is labelled as face P and its complementary half is labelled E.

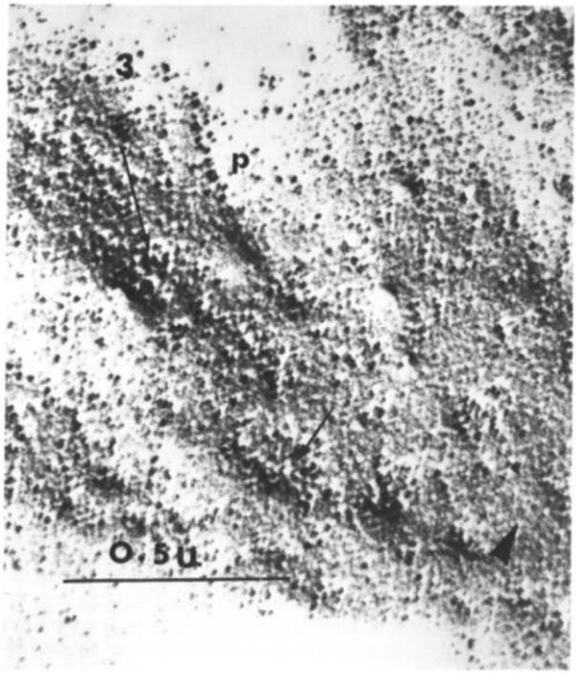
3.1. *Non-synaptic zone of innervated sarcolemma*

The fractured face P of the sarcolemma in control muscle is characterized by numerous particles which are mostly 80 Å in diameter (fig.1 A). The particles are rather uniformly dispersed and their density is approximately 2000/μm^2. The orthogonal arrays similar to those described by [8] are also of common occurrence. There are also numerous depressions of 300–500 Å diameter which are presumably openings of cortical vesicles or T-tubules. Fractured face E shows numerous protuberances that are complemen tary to the 300–500 Å depressions on face P. This face shows far fewer 80 Å particles than on face P.



A

B



C

D

3.2. *Non-synaptic zone of denervated sarcolemma*

The replicas prepared from denervated muscle show marked changes in the structure of the fractured face P of the sarcolemma as compared to that on the corresponding face in the control muscle. Some of these fractured faces are conspicuous by the presence of aggregates (figs.1C,D) of particles which are mostly 110 Å to 180 Å in size. The number of particles in each aggregate varies from 4–50 or more particles. These aggregates are randomly dispersed over the P face; but the number of such aggregates varies in replicas. It is apparent that such aggregates of particles are not discerned on either of the fractured faces of the sarcolemma in the control muscle. Also the size of these particles is different from the particles in the control muscles. Moreover, the 80 Å particles that are predominant on the P face of the sarcolemma of the control muscle are reduced in number. Many fractured faces where aggregates are not observed, the particles are randomly distributed and have packing density of 400–1000/μm^2 (fig.1B) as compared to 2000/μm^2 in those of control.

The E face of the denervated sarcolemma is relatively unchanged, there being no groups of depressions complementary to the aggregates on the P face. Such an asymmetry may result from a preferential attachment of the particles to the cytoplasmic half of the membrane as has been described for intramembranous particles representing bacteriorhodopsin in the purple membranes of halobacteria [9].

4. Discussion

The above studies indicate that denervation induces the formation of aggregates of large particles. The size of many of these particles in the aggregates is consistent with that reported for Ach receptors by Rash and Ellisman [8] in mammalian neuromuscular junction. It is likely that these particles represent the sites for known extrajunctional Ach sensitivity in denervated muscle. Work is in progress to test this hypothesis. The observed variation in the size of the aggregates of particles may be related to a gradual and progressive change in the membrane. The significance of the apparent decrease in number of 80 Å particles on face P is uncertain.

Acknowledgements

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Fig.1. (A) Freeze-fractured innervated sarcolemma (control) showing the convex face (P) of the membrane. The intra-membranous 80 Å particles are uniformly dispersed over the entire face. Arrow head indicates direction of shadowing in all figures. × 60 000. (B–D) Freeze-fractured sarcolemma from denervated muscle showing various alterations on face P. (B) Shows a marked decrease in number of 80 Å particles as compared to those discerned on this face in the control (A). The appearance of aggregates of particles which are 110–180 Å is seen in (C) (arrows). Larger aggregates of such particles are seen in (D) (arrows). The face labelled E in (B) is the concave face which shows 300–600 Å protuberances representing openings of T-tubules or cortical vesicles. A few 80 Å particles are also seen on this face, (B–D). × 60 000.