Scanning Electron Microscope Studies on the Schwann Cells in Rat Motor Endplates with Special Reference to their Finger-like Projections

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Summary. Three-dimensional structures of the Schwann cell in the rat motor endplate were observed by scanning and transmission electron microscopy. A few Schwann cells lodged over the surface of the motor endplates and their nuclear portion swelled roundly. From the Schwann cell body, a few processes extended downwards, divided several times and covered only the upper orifice of the synaptic groove; they did not cover the surface of the protuberances or the ridges of the sarcolemma which separated the synaptic grooves. The lateral sides and ends of the Schwann cell processes were frilled with finger-like projections. They fixed on the surface of the basal lamina of the sarcolemma and sealed the nerve terminals in the synaptic grooves.

Scanning electron microscope (SEM) studies on the motor endplates have been reported after removal of connective tissue components by collagenase digestion (Shotton et al., 1979) or by HCl digestion (Desaki and Uehara, 1981). Using the HCl hydrolysis method, we have been studying the structural differences in the motor endplates of the red, white and intermediate fibers of the rat (Ogata and Kubota, 1982; Ogata and Yamasaki, 1984), and in the course of this investigation we found some peculiar structures of Schwann cells (teloglia) in the motor endplate, especially the terminal parts of their processes showing finger-like appearance. The findings will be depicted and discussed in this paper, comparing with the transmission electron microscope (TEM) observations.

MATERIALS AND METHODS

Male Wistar rats weighing 200-250 g were used. The intercostal muscles were cut into small blocks (2 x 1 x 1 mm) and fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 2 hrs. They were washed in the same buffer and treated with 8 N HCl for 20-60 min at 60°C (Desaki and Uehara, 1981). They were dehydrated through a graded series of ethanol and immersed in isoamyl acetate for 30 min. After being dried by the critical point method, the specimens were impregnated with osmium (Kubotsu and Ueda, 1980), then slightly coated with platinum (about 5 nm in thick-
ness). They were examined under a field emission type SEM, Hitachi S-700 with accelerating voltage at 15 kV.

For the comparative TEM study, the intercostal muscles were fixed in 2.5% glutaraldehyde and 2.0% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.4) for 2 hrs and postfixed in 1.3% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.4) for 2 hrs. The tissues were dehydrated through a graded series of ethanol and embedded in Epon. Thin sections were stained with uranyl acetate and lead hydroxide and examined under a JEOL 100S TEM.

RESULTS

In the specimens, where the connective tissue components were adequately removed, the Schwann cells and the nerve terminals (terminal branches) remained on the surface of the muscle fibers (Fig. 1–3). The nerve terminals extended in the primary synaptic grooves and several times dichotomously ramified. Along the course or at the terminals, small spherical swellings of the nerve terminals were seen (Fig. 1). These

Fig. 1. SEM image of a motor endplate of a white fiber. Nerve terminals (NT) lie in the synaptic grooves. The small spherical swellings (S) fit in the hemispherical concavities (C) of the synaptic grooves. They connect with a slender stalk (arrows) to the nerve terminals. The nuclear portions of the Schwann cell bodies (SC) appearing as spherical bulges and their processes extend downwards and divide several times. Note that the surface of the upper orifice of the synaptic grooves is covered by Schwann cell processes (SP), whereas the small ridge (R) with slit-like openings of junctional folds is not covered by them. ×7,000
Fig. 2. SEM image of a motor endplate of an intermediate fiber. The Schwann cell (SC) processes cover the upper orifice of the synaptic grooves (arrows), but do not cover protuberance (P) of the sarcolemma. Their sides and ends are provided with finger-like projections. A process of a Schwann cell crosses over that of another cell (arrowhead). × 5,000

Fig. 3. A closer view of a part of Figure 2. Note the finger-like projections of the Schwann cell attach to the surface of the sarcolemma (SL). Arrows show slit-like openings of the junctional folds. × 20,000
swellings connected with a slender stalk to the nerve terminals and fitted in the semi-spherical concavities of the primary synaptic grooves. A few Schwann cells lodged over the surface of the motor endplate; their nuclear portion or cell body swelled roundly (Fig. 1, 2). From the cell body, a few cytoplasmic processes extended downwards and divided several times. These Schwann cell processes covered only the upper orifices of the synaptic grooves, which contained the nerve terminals, but did not cover the protuberances of the sarcolemma which separated the synaptic grooves (Fig. 2). Occasionally, the process of one Schwann cell crossed over that of another Schwann cell (Fig. 2). The lateral sides and ends of these processes, small finger-like projections were seen. At higher magnification, these projections measured 0.4-3.0 μm in length and 0.14-0.2 μm in diameter (Fig. 3). Some of them were palm-like in shape with several fingers. The finger-like projections closely adhered the surface of the sarcolemma, so the Schwann cell processes sealed the nerve terminals in the synaptic grooves.

In the specimens in which the nerve terminals have been detached, slit-like openings of the junctional folds were seen on the surface of the synaptic grooves as well as on the surface of the small ridges of the sarcolemma which separated the synaptic grooves (Fig. 4). As seen in Figure 1, such small ridges were not covered by Schwann cell processes, although adjacent upper orifice of synaptic grooves were covered by them. Such uncovered small ridges with slit-like openings of the junctional folds were frequently seen throughout in the motor endplates of the white fiber, moderately in the intermediate fiber but usually lacking in the red fiber.

The TEM observations indicated that the nuclear portion of the Schwann cell body lodged over the surface of the motor endplate and its cytoplasmic processes extended downward (Fig. 5). These processes became rather flat in shape and covered the surface of the upper orifices of the synaptic grooves, which contained the nerve terminals (Fig. 5, 7). However, they did not cover the small elevated area of the sarcoplasm which contained numerous mitochondria (Fig. 7). Numerous junctional folds were seen not only in the area of the synaptic grooves but also in the small ridges unassociated with nerve terminals and uncovered by Schwann cell processes. TEM images confirmed that the terminal parts of the Schwann cell processes were palm-like in shape with several finger-like projections closely adhering to the basal lamina of the sarcolemma (Fig. 6).

![Fig. 4. SEM image of a nerve detached specimen of a white fiber. Slit-like openings of the junctional folds are seen on the surface of the synaptic grooves (G) as well as on the surface of the small ridges (R). ×18,000](image_url)
Fig. 5. TEM image of a motor endplate of a white fiber. From the nuclear portion of the Schwann cell body (SC), a few processes (SP) extend downward and cover the surface of the nerve terminals (NT). x 6,000

Fig. 6. Higher magnification of a part of Figure 5. Terminal part of Schwann cell process (SP) shows palm-like structures with several finger-like projections (arrows). Note that the tips of the fingers closely adhere to the basal lamina of the sarcolemma (arrowhead). x 12,000
DISCUSSION

The Schwann cells in the motor endplate of the vertebrates were examined by SEM after removal of connective tissue components by HCl hydrolysis. The profiles of nerve endings or, more exactly, those of Schwann cells investing the terminal axons are exposed on the surface of the muscle fiber and the nuclear portions of the Schwann cells are roundly swollen along nerve endings as demonstration by DESAKI and UEHARA (1981). In their report, however, information on more precise relationship between the Schwann cell processes and synaptic grooves or nerve terminals was lacking. It has been generally believed that the Schwann cell processes covered the entire surface of the motor endplate. From the present study, it was elucidated that the Schwann cell processes covered only the upper orifices of the synaptic grooves, but did not cover the protuberances or the ridges of the sarcolemma in the motor endplate.

From the TEM observations of the motor endplate of the rat soleus muscle, exposed junctional folds unassociated with axonal terminals and uncovered by Schwann cell processes were reported by CARDASIS and PADYKULA (1981). They stated that such exposed junctional folds are associated with either focal denervation or reinnervation and that motor endplate renewal occurs in the adult muscle. However, from the present observations, slit-like openings of the junctional folds were seen on the surface of the synaptic grooves as well as on the surface of small ridges which separated synaptic grooves. Furthermore, the surface of small ridges was not covered by Schwann cell cytoplasm. And such small ridges with junctional folds were frequently seen throughout in the motor endplates of all white fibers and moderately seen in all intermediate fibers examined so far. The exposed junctional folds seemed to exist in...
all normal state motor endplates in the white and intermediate fibers. Probably most of them not related to focal denervation or reinnervation. Such exposed junctional folds were usually lacking in the red fibers, but precise three-dimensional structural differences of motor endplate in three types of fibers will be reported in a separate paper.

It is well known that the finger-like projections of Schwann cells appear at the node of Ranvier (Krstić, 1978). From SEM observations of the frog motor endplate, small lateral projections of Schwann cell investments which showed finger-like or knob-like appearance were reported by Desaki and Uehara (1981). They stated that these projections did not appear to be artitectural structure, and suggested that further studies, including reconstruction from serial sections, might be needed to ascertain whether these projections are genuine structures. In the present study, these finger-like projections were constantly observed in well preserved specimens of rat motor endplate by SEM and could be confirmed by TEM. TEM observations revealed that these finger-like projections closely adhered on the surface of the basal lamina of the sarcolemma. It seemed reasonable to postulate that they might play a role in fixing the Schwann cell processes to the basal lamina of the sarcolemma and in sealing the nerve terminals in the synaptic grooves.

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