MORPHOLOGICAL REMODELING OF THE MOTOR END PLATE IN RAT SOLEUS MUSCLE AFTER LIMB IMMOBILIZATION BY CASTING

Wai-Yi Wang and Keh-Min Liu

The effects of muscular inactivity on motor end plate(MEP) structures of the rat soleus muscle were studied qualitatively and quantitatively. Eight adult rats were divided into two equal groups. One group had their right hind limbs immobilized by application of plaster casts. After 14 days in the casts, the soleus muscles of all the animals were removed. Three groups of MEPs were analyzed to compare their structural characteristics: the immobilized group (the MEPs of the casted soleus muscles), the contralateral group (the MEPs of soleus muscles which were in legs contralateral to the casted legs), and the control group (the MEPs of normal soleus). The MEPs were examined in a teased preparation of soleus muscles stained by silver-impregnation. The architecture of the MEPs was measured by an "AMS VIDS Ⅲ Image Computerized System" which was mounted with a light microscope for morphological analysis. The measured parameters were the structural elements of MEPs.

The results of the present study showed that: MEPs of the immobilized group exhibited more complex and highly branches structures than the other two groups. The total number of branches and the length of the terminal perimeter in the immobilized group increased significantly. Concomitantly, the amount of swollen terminals of this group were obviouly larger than in the other two groups. The results demonstrated that the muscular inactivity of the casted limbs produced denervation-like changes at branches of nerve terminals. These changes were the result of altered muscular function, and led to structural remodeling of MEPs of the muscles.

Key words: motor end plate, limb immobilization

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The motor end plate (MEP) is the final pathway of the motor nerve system. During the development of animals, there is a process of growth and degeneration of nerve terminals within the MEPs[1-3]. This process appears to constantly remodel the structures of the MEP, and it plays an important role in the ability of the nervous system to recover from damage, store information and maintain the nerve terminal population.

During senescence, there is a changeable incidence of growth and degeneration of motor nerve terminals. A wide variety of studies[4-8] have shown that "muscular activity" seems to govern these dynamic remodeling processes. "Muscular inactivity" is one of the experimental models used to study the factors regulating motoneurone properties.

In the present study, the immobilized limb procedure produced temporary inactivity of muscles. This method concomitantly produces the anatomical completeness of nerve innervation[6,7,9]. We observed the MEPs of soleus muscles immobilized by casting. The MEPs were examined by light microscope, and a computer program was used for morphometry of these MEPs. Such a qualitative and quantitative study could be an essential background for appraising pathologic changes in the neuromuscular system.

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MATERIALS AND METHODS

Experimental preparation

Eight female adult rats with body weight ranging between 200-250 gm were divided into two equal groups. They were housed in the same room on a 12-h light-dark cycle at 20°C. Food and water were supplied ad libitum. One group of rats had their right hind limbs immobilized by application of plaster casts with knees and ankles fixed in full extension and plantar flexion, respectively. The immobilized limbs were casted for 2 weeks.

After 14 days in casts, all the animals were anaesthetized with ether and the soleus muscles were removed. The intramuscular nerves and MEPs were examined in a teased preparation of muscles stained by silver-impregnaton as described by Barker and Ip[10,11]. This staining method gives a clear, complete picture of the terminal innervation, and makes it possible to determine the exact points of origin and termination of fine outgrowths of the terminal axon.

The MEPs of soleus muscles were selected for analysis on the basis of clarity. They were compatible with the following criteria: (1) The complete nerve terminal arborization was fully incorporated within the section of tissue; (2) The orientaion of the MEPs was parallel to the plane of the section; (3) More than 95% of its area had to be clearly visible[12-14]. The morphological parameters evaluated within each MEP are shown in Fig. 1.

Once the nerve terminals were selected and studied by a light microscope, the images were superimposed onto an AMS VIDS Ⅲ morphometric system. The VISD Ⅲ is a resolutive semi-automatic image analysis system. It utilizes a monitor to provide direct imaging of samples viewed with the microscope. Drawings were traced on the monitor screen and the parameters of each MEP were computerized quantitatively.

Statistical analysis

Three main groups of MEPs were compared: the control group (the MEPs of normal soleus muscles), the immobilized group (the MEPs of the immobilized soleus muscles) and the contralateral group (the MEPs of soleus muscles which were in leg contralateral to the casted legs). 15 measurements were obtained from each soleus muscle, for 60 measurements per group. There was no difference in the morphology of MEPs from rat soleus muscles of the same age, and the samples of each group were therefore pooled.

These data were statistically analyzed using one way analysis of variance (ANOVA) to compare the mean values of all variables in the three groups of MEPs. Scheffe's procedure was used to identify the differences among groups. The probability value for significance was selected to be $p < 0.05$.

RESULTS

The axon terminals in the normal soleus muscles were delicate and typically ended in fine tapers (Fig. 2a). Sprouting terminals were found within some normal MEPs (Fig. 2b). We examined the MEPs of the immobilized group,

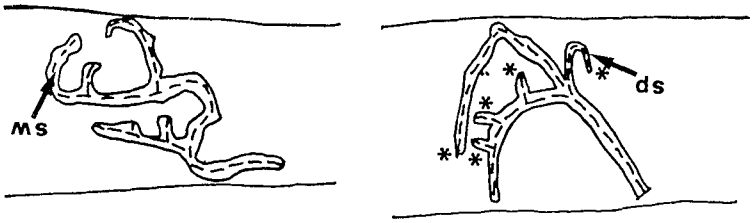

Fig. 1. Drawing of a light micrograph of the motor end plate in rat soleus muscle.
Perimeter: outermost solid line.
Terminal area: area within the outermostline.
No. of branches: each shown as a dashed line within the perimeter (*).
Branch length: sum of lengths of all dashed lines.
SW: swollen branch.
SP: sprouting branch.

and found that the most striking features were the more complex and highly branched configurations (Fig. 3a, e). Sprouting terminals were noted in some MEPs of the immobilized and contralateral groups (Fig. 3b). Moveover, abundant swollen and irregularly shaped terminals were found as the other obvious features of these two groups of MEPs. (Fig. 3c, d, e).

These changes were quantitatively analyzed. The results are listed in Table 1. The data depicted significant increases in the number of branches and the nerve terminal perimeters of the immobilized group when compared to either the contralateral group or control group ($p<0.01$). However, the total branch length and terminal area were not statistically different among the three group of MEPs. In contrast, the ratio of terminal area to perimeter in the immobilized group was decreased significantly when compared to the other two groups

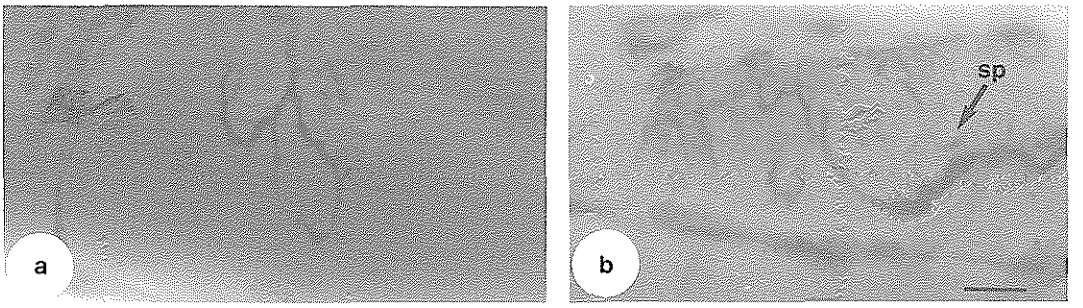

Fig. 2. Motor end plates in soleus muscles of control group. sp: sprouting branch. calibration bar: 20μm.

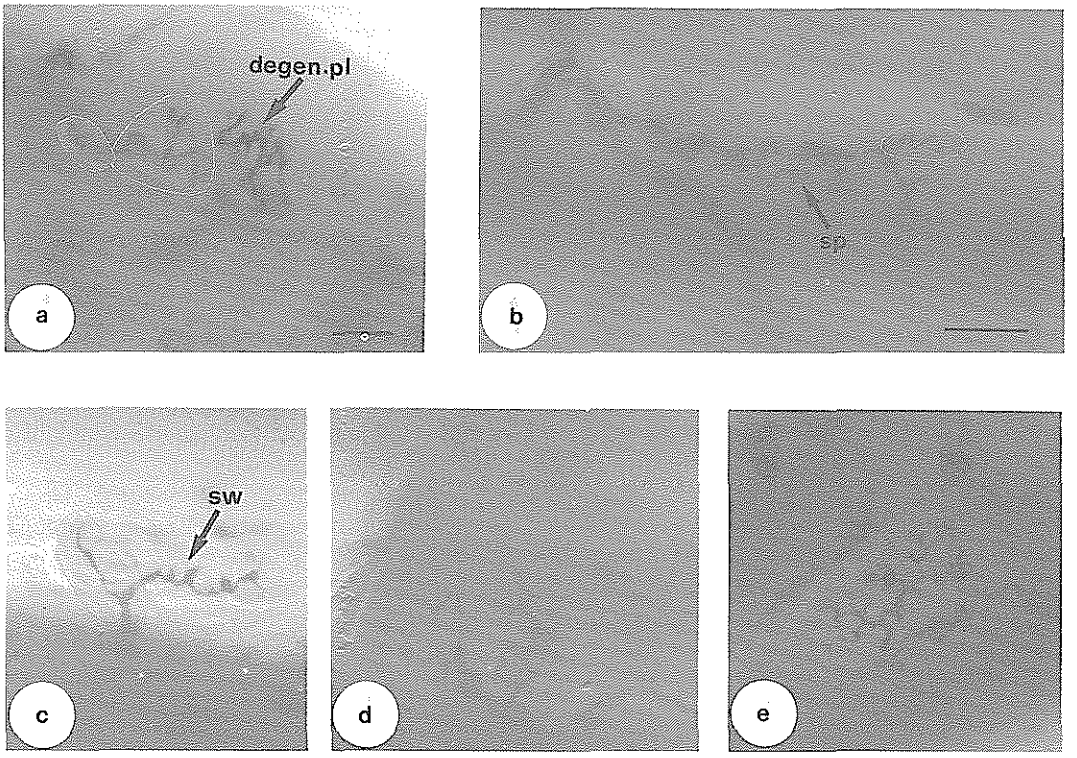

Fig. 3. Motor end plates in soleus muscles of disused group. sp: sprouting branch. sw: swollen branch. degen. pl: end plate was separated from the axon and degenerated. calibration bar: 20μm Fig. 3a, c, d, e: ——— Fig. 3b: ———

Table 1. Quantitative Analysis of MEPs Structure in Teased Muscle Preparations of Control, Immobilized and Contralateral Groups

Parameter	Control n=60	Contra. n=60	Immobili. n=60
No. of branches	6.0±2.2	5.5±1.8#	8.1±2.8*
Total branch length (μm)	84.1±23.3	76.9±24.9	102.6±98.3
Area (μm^2)	134.1±45.1	114.6±43.6	129.7±51.7
Perimeter (μm)	165.4±45.0	151.0±48.4#	186.8±70.1*
Ratio of area to perimeter	0.80±0.17	0.76±0.14#	0.70±0.13*
No. of sprouts	0.12±0.33	0.19±0.39	0.22±0.50
No. of swollen branch	0.09±0.39	0.49±1.17	1.07±1.76

1. Contra.: contralateral, Immobili.: immobilized
2. Data are means±SE per MEP from 180 muscle fibres obtained from eight animals.
3. * Significant difference ($p<0.01$) between immobili. and control groups.
   # Significant difference ($p<0.01$) between immobili. and contra. groups.

($p<0.01$).

DISCUSSION

This was a qualitative and quantitative study on the alterations of MEP features following limb immobilization. The AMS VIDS Ⅲ system allows for computable comparison of the parameter valves of images and thus minimizes error associated with artifical algorithms. The measurements of the MEPs were liable to error through shrinkage and deformation of the material during preparation, but since all the measurements were made on material prepared in the same way, any comparisons made within the results were valid.

The results of this study demonstrated that limb immobilization induced a number of alterations in the structures of the MEP. Increased arborization, complexity and degenerative nerve terminals were observed. Thus it is suggested that these MEPs were in a state of remodeling.

Parameters of the nerve terminal area and perimeter were characteristic of the size of MEPs[1,2]. In the present study, the terminal area and perimeter of the contralateral group were obviously decreased. These results might be due to the decreased physical activity of both legs of experimental rats.

Although the terminal area of immobilized MEPs was larger than in the contralateral group, the distribution of these areas was more diffuse. This is because the ratio of the terminal area to the terminal perimeter is a measurement of the degree of tortuosity of the nerve terminal outline[8,15]. Because of the significant decreased ratio value in the immobilized group, it suggested, the distribution of the terminal area of immobilized MEPs was more regional and heterogeneous.

The increased in number of branches per end plate in the immobilized group was due to the increased incidence of sprouting and degenerative branches. It is thought that sprouts are evidence of growth of the terminal axon and referred to as "growth configuration"[6,16]. Terminal sprouting has been reported in studies of reduction of muscle activity and was observed during aging[15,16]. Swollen axons are described as a characteristic of nerve terminal degeneration[11,16,17]. The increased amount of sprouting may serve to replace terminals as they are damaged or lost, and thus acts to maintain the structural integrity of MEPs.

Such remodeling with axonal terminal sprouting and degeneration is a morphological basis for physiological change[15]. It may be related to variation in functional demands on workload. Currently, degeneration is suggested to lead to a decrease in the effective area of synaptic contact.

In the present study, evidence of alterations were at the presynaptic level. They showed that muscular hypoactivity led to increased swollen MEP terminals. The larger number of branches would contribute to increased transmitter re-

lease to maintain synaptic transmission.

The obvious clinical relevance is that since limb immoblization is used popularly for the treatment of fractured bones and injured tissues in human patient, the interaction between muscles and nerves under such clinical conditions should be examined.

In conclusion, the present study revealed that limb immobilization by casting altered the morphology of the MEPs in rat soleus muscles.

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石膏固定後大白鼠比目魚肌神經運動終板的形態改變

王慧儀　劉克明

本實驗是研究大白鼠的下肢經石膏固定後，下腿的比目魚肌內「神經運動終板」的形態改變；並對這些改變作定性及定量的分析。

本實驗採用8隻成熟的雌大白鼠，平均的分作2組。一組作控制組，另一組動物的右側下肢以石膏繃帶纏繞包紮。經石膏固定14天以後，取下所有動物的比目魚肌，並比較以下三組比目魚肌中的運動終板的結構：固定組（石膏固定的比目魚肌）、對側組（實驗組老鼠左腳的比目魚肌）及控制組（正常的比目魚肌）。肌肉經銀浸透法染色後，藉分離處理把肌纖維分離開來。用光學顯微鏡觀察分離後的肌纖維，可清楚觀察到支配在纖維上的神經末梢及運動終板。在光學顯微鏡下的影像，被傳送並投射在一與顯微鏡相連接的「AMS VIDS Ⅲ電腦化形態分析器組」的電腦銀幕上。透過此電腦器組的運作，可計算出實驗所要分析之影像結構的實際數值，以此研究運動終板結構特徵的變化。

實驗結果顯示，固定組的運動終板結構較複雜，且分枝繁多；此組終板內的總分枝數、終板的週邊長度，在統計學分析上，均較控制組及對側組的有顯著的增加。同時，在比較三組終板內的腫大分枝數時，固定組的數目也明顯的多於其他兩組。

這些結果顯示利用固定肢體以限制肌肉的活動，會造成運動終板的退化性變化；而運動終板的這些改變正是因應肌肉功能改變下的一種結構上的調節及塑變。

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