Effect of Denervation on Neuromuscular Transmission and Glutamate Sensitivity of the Locust Wing Muscle Fibers

S. V. IVLEV[1], G. P. PAPIDZE[2] and Yu. E. MANDELSHTAM[1]

1 *Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, M. Thorez Ave. 44, 1944233 St. Peterburg, Russia*
2 *Biological Faculty, Tbilisi Javakhishvili State University, I. Chavchavadze Ave. 1, 380028 Tbilisi, Georgia*

Abstract. Changes in neuromuscular transmission and ultrastructure of synapses taking place after motor nerve cutting during 20 subsequent days were studied on the wing muscle of the locust (muscle 120) using intracellular recording from phasic fibres. The membrane potential (MP) and the excitatory postsynaptic potential (EPSP) showed practically no changes in comparison with the controls through day 8 after denervation. After the development of a neuromuscular transmission block between days 8 and 14, EPSP and miniature EPSP (MEPSP) disappeared, and the MP level somewhat decreased in comparison with controls. MEPSP frequency and amplitude increased in the first days after denervation. The sensitivity to glutaminic acid (Glu) gradually increased after nerve cutting, reached maximum on day 8 and remained at the same level subsequently (threshold concentration 0.003 mmol/l vs 0.3 mmol/l in the control). At the same time, the maximum amplitude of depolarization produced by Glu increased 1.5–2 times but slightly decreased subsequently. Beginning from day 4 after denervation synaptic vesicles began forming separate groups. On day 14 vesicles were grouped in macroconglomerates at a distance from the presynaptic membrane. On day 20 no axon profiles could be detected. The results suggest a similarity of denervation changes in insect wing and leg muscles.

Key words: Denervation — Neuromuscular transmission — Glu sensitivity — Ultrastructure of synapses — Insect wing muscle

Introduction

The properties of glutamate reception have been studied on neuromuscular synapses of locust (Usherwood and Machili 1968; Usherwood 1974; Piek 1985) and in fly larvae (Jan and Jan 1976). An increased sensitivity of synaptic D-receptors

for glutamate has been shown in the muscular fibers of extensor tibia (Usherwood 1973) and retractor unguis (Mathers and Usherwood 1978) of the metathoracal leg of locust. The properties of glutamate receptors in fast muscles involved in the activity of the wing system have been much less studied (Alexandrov and Baranov 1984). It is known that phasic and tonic fibers in the wing muscles differ from each other in their parameters of glutamate response and in miniature excitatory postsynaptic potentials (MEPSP) (Grigorev 1980; Grigorev and Mandelshtam 1981; Mandelshtam 1983). However, changes of the excitatory postsynaptic potentials (EPSP), MEPSP and ultrastructure of the neuromuscular synapses in wing muscles after motor nerve cutting have remained largely unknown.

Materials and Methods

Experiments were performed on phasic fibers of the wing muscle (muscle 120 by the Snodgrass nomenclature, Snodgrass 1935) of the locust (*Locusta migratoria migratorioides* R.V.). Separately maturing males were used for experiments. The insects were kept in a hothouse under day-and-night lighting at a temperature of $28 \pm 1\,^{\circ}C$, and they were given green feed.

Motor nerve (4^{th}) was cut near the metathoracal ganglion from the ventral side of the thorax through a small incision. The chitin plate was then returned to its place. Approximately 85–90% of the individuals undergoing denervation survived.

Muscles for the electrophysiological investigations were taken on days 1 – 4, 8, 14 and 20 after denervation; for electron microscopic examination samples were taken on days 4, 8, 14 and 20. Muscles from an intact group of insects were used as a control. Controls were kept under the same conditions as the denervated insects.

The muscle was completely isolated from the insect body and placed into a perfusion chamber. The physiological solution flown through the chamber had the following composition (in mmol/l): NaCl, 135; KCl, 3.0; $CaCl_2$, 2.0; $MgCl_2$, 1.6; KH_2PO_4, 3.0; Na_2HPO_4, 4.0. The solution was used at room temperature and had a pH 6.8.

In experiments with nerve stimulation, the $CaCl_2$ content was reduced to 1.4 mmol/l, and 25–30 mmol/l $MgSO_4$ was added to abolish muscle contraction.

Changes of EPSP, magnitude and frequency of MEPSP, sensitivity of muscle fibers to glutamic acid (Glu) as well as ultrastructure of neuromuscular synapses after denervation were studied. Glutamate responses, MEPSP and EPSP were recorded by intracellular glass microelectrodes filled with 2 mol/l potassium citrate solution; their resistance was 15–20 MOhm. To obtain EPSP, the motor nerve was stimulated by a series of 5 electric pulses with a recurrence frequency of 100 Hz, via a suction electrode.

Glutamate was administrated to the surface of the muscle fibers from a glass micropipette with a tip diameter of 100–200 μm, using the method of microsuperfusion (Gapon et al. 1978).

For ultrastructural investigation, a slightly extended muscle previously fixed in the insect body for 3–5 minutes was excised and placed into fixation solution of the following composition; 2.5% glutaraldehyde, 0.05 mol/l sodium cacodylate, 0.2 mol/l saccharose, pH 7.4; $T = 4\,^{\circ}C$. Washing was carried out with cold 0.05 mol/l cacodylate buffer, which contained 0.34 mol/l saccharose. After fixation in 1% OsO_4 solution during 10 minutes, washing with cold cacodylate buffer and dehydration in alcohol series and acetone, the

tissue was flooded with araldite. Sections were cut on an ultratome (LKB–3) and contrasted with alcoholic solution of uranyl acetate and lead citrate according to Reynolds (1963). The samples were examined in electron microscope JEM-100B.

Results and Discussion

Intact muscle fibers had a membrane potential (MP) of -51 ± 10 mV. In contrast to the data obtained by Usherwood who has observed decreasing MP values during the first day after denervation, in our experiments there was effectively no difference in MP values between denervated muscle fibers and control animals up to day 14. On day 20 after denervation MP value was somewhat higher (Table 1).

On day 1–2 after the nerve cutting, the form and the magnitude of EPSP did not differ from those recorded from control muscles. Local contractions were observed since day 3 after penetrating the fiber membrane by a microelectrode. This phenomenon was seemingly the result of increasing excitability of denervated muscles. To abolish these contractions, $MgCl_2$ concentration had to be raised up to 30 mmol/l. In our experiments the EPSP and MEPSP disappeared on day 14 after denervation. On day 20 the picture remained unchanged.

An increase in sensitivity of denervated muscle fibers to Glu was observed since the third day. In the control Glu depolarised the fibers membrane at concentrations as low as 0.03 mmol/l, while after denervation in some fibers a slight depolarisation was observed in response to 0.01 mmol/l Glu already on day 2 (Table 1). On subsequent days the sensitivity varied but was always higher than in the control. The highest sensitivity to Glu was observed on day 8 when the threshold concentration was 0.001 mmol/l. The variation of Glu sensitivity of fibers of one and the same muscle may probably be explained by different rates of the degenerative processes in motor axon terminals. Muscle 120 is rather large and the length of the individual axons can determine inequal time of morphological changes occurring in motor terminals.

The average maximal amplitude of Glu responses began to increase from day 2 in comparison with the control. On day 8 after denervation the maximal amplitude reached 20 mV at Glu concentration of 0.1 mmol/l. Later, the maximal amplitude tended to decrease but remained higher than the control (Table 1). Partial desensitization of responses to Glu application appeared on day 4–8 after the nerve cutting. The amplitude of the responses reached a maximum and then gradually decreased to a certain level, although Glu application was not stopped (Fig. 1).

In intact muscle fibers MEPSP amplitude varies in a wide range and its maximal value reaches 270–340 μV. Possibly, these fluctuations are not only connected with the number of quanta transmitter released from the synapse but also with the multiterminal innervation of the insect muscle and with the different distance between the microelectrode and the individual motor terminals.

Table 1. Neuromuscular transmission blockade and changes of membrane potential (MP) and responses to glutamate acid (Glu) in phase fibers of locust wing muscle in the process of denervation.

	Control	Days after denervation						
		1	2	3	4	8	14	20
EPSP and MEPSP	yes	yes	yes	yes	yes	yes	yes	yes
MP (mV)	-56 ± 8 $n = 17$	-51 ± 8 $n = 17$	-51 ± 8 $n = 21$	-50 ± 11 $n = 18$	-52 ± 10 $n = 13$	-51 ± 9 $n = 13$	-56 ± 11 $n = 11$	-46 ± 5 $n = 16$
MP (mV) control, on the same days			-51 ± 10 $n = 7$				-61 ± 4 $n = 6$	-58 ± 8 $n = 4$
Maximum amplitude (mV) of response to Glu at concentration (mmol/l)	8.5 ± 1.5 $n = 6$ 0.3–1.0	8.5 ± 1.5 $n = 6$ 0.3–1.0	11.5 ± 3.0 $n = 8$ 0.3	12.0 ± 2.5 $n = 4$ 0.3	14.5 ± 4.5 $n = 7$ 0.1–0.3	17.5 ± 4.0 $n = 7$ 0.1–0.3	15.0 ± 2.5 $n = 7$ 0.1–0.3	10.5 ± 2.5 $n = 8$ 0.1–0.3
Threshold concentration of Glu (mmol/l)	0.03		0.01–0.03		0.01	0.003–0.01		

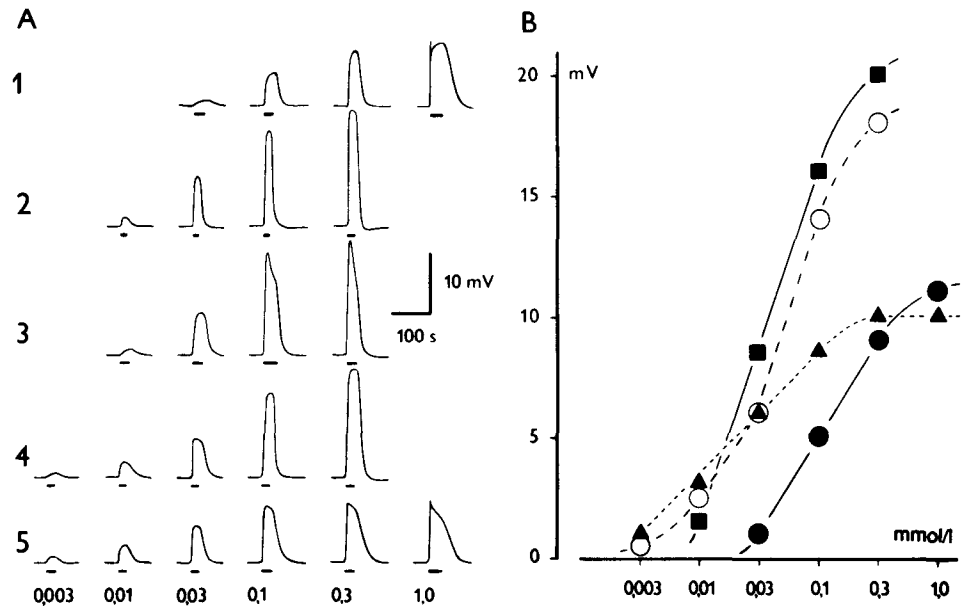

Figure 1. Change of depolarization responses to Glu; muscle fibers after denervation. *A*: Recordings of responses to microsuperfusion application of Glu. Control (1) and days 4, 8, 14 and 20 (2,3,4, and 5, respectively) after motor nerve cutting. The figures indicate Glu concentrations in mmol/l. Application period is indicated by the bold line under each recording. Partial desensitization of responses to Glu after motor nerve cutting is well seen in A3 and A5. *B*: Dose – effect curves for the responses of muscle fibers; control and denervated fibers. Filled circles, control; squares, day 4; empty circles, day 14; triangles, day 20 after denervation.

After motor nerve cutting the pattern of MEPSP amplitude distribution changed already on day 2 (Fig. 2). If, in the intact muscle fibers. the low-amplitude (less than 100 μV) MEPSP were generated with the most probability, then on day 2 after denervation the probabilities of MEPSP appearance of small and medium amplitudes (100–200 μV) became similar. Moreover, the frequency of the onset of high-amplitude (more than 200 μV) MEPSP was sharply increased and infrequent "giant" MEPSP with an amplitude of 0.5–2.0 mV were observed. On subsequent day a partial restoration of the distribution of MEPSP amplitudes was observed, however, a portion of high-amplitude MEPSP remained increased until their disappearance after day 8 (Fig. 2). The frequency of MEPSP strongly varied in different fibers both in intact and denervated muscles. Nevertheless, after nerve cutting a tendency to a gradual increase of frequency of MEPSP generation was prominent from day 2 through 8 (Fig. 2). Both EPSP and MEPSP disappeared on day 14 after denervation (Table 1).

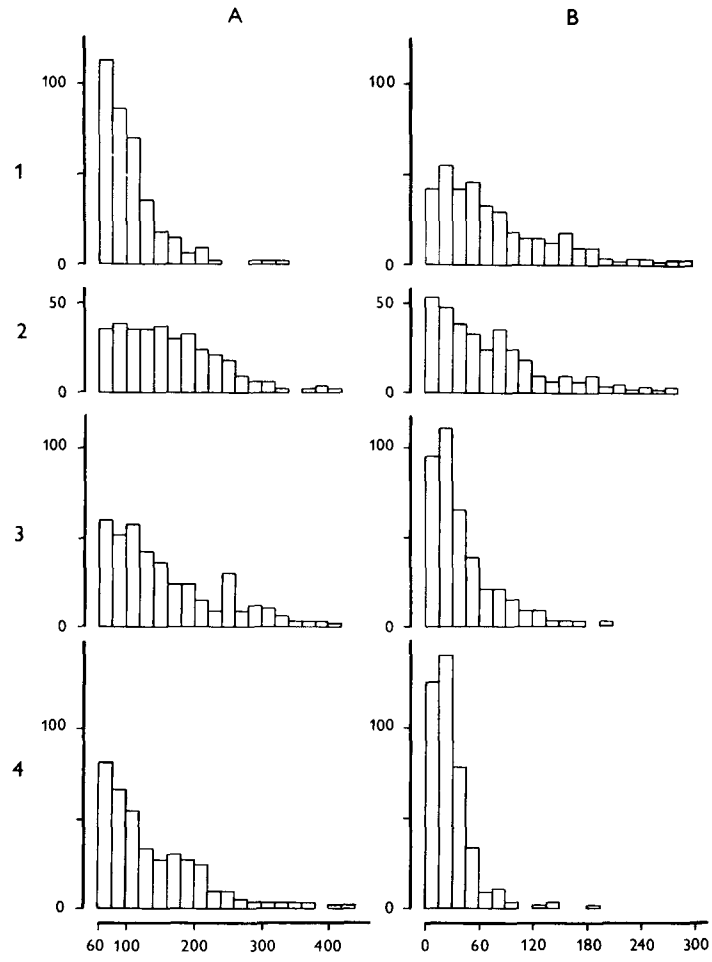

Figure 2. Histograms of distribution of miniature EPSP by amplitude (A) and by duration of inter-pulse intervals (B) in controls and after denervation. 1, control; 2, 3, and 4, postdenervation days 2, 4 and 8. Abscissa: A – MEPSP amplitude in μV, B – duration of interpulse intervals in ms. For each histogram, $n = 400$. For better comparison the results presented were obtained from muscle fibers with the same MP (−60 mV).

The appearance of "giant" MEPSP was seen previously in denervated fibers of leg muscles of the locust (Usherwood 1963 a,b; 1973). The appearance of such MEPSP in our experiments suggests a similarity of degenerative processes that occurred after denervation both in slowlier leg muscles and in fast wing muscles of insects.

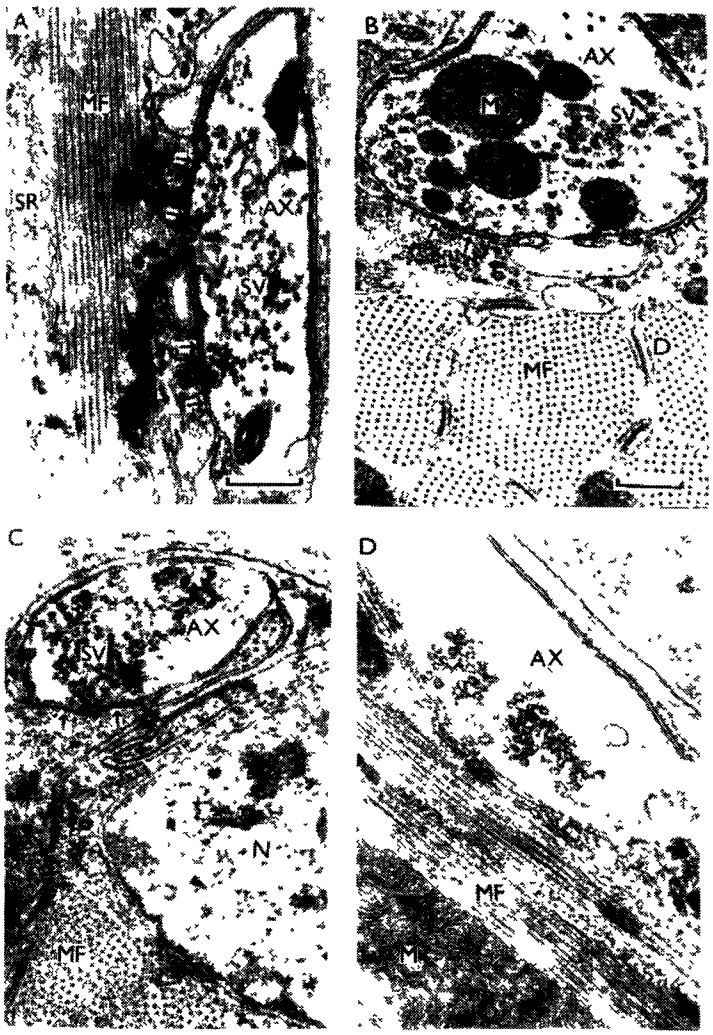

Figure 3. Ultrastructure of neuromuscular synapses of intact phasic fibers of muscle 120 and at different postdenervation periods. A, B, C, D – controls and postdenervation days 4, 8 and 14. Zone of synaptic contacts (arrows), axon (AX), synaptic vesicles (SV), sarcoplasmic reticulum (SR), myofibril (MF), mitochondrion (M), nucleus (N), dyad (D). Scale: for A – 0.5 μm, B, C, D – 0.3 μm.

We studied the structure of neuromuscular synapses located on phasic fibers of muscle 120. All phasic fibers are innervated by fast type axons (Grigorev 1980), and they do not differ from each other by their structure of neuromuscular synapses. There are numerous synaptic vesicles with a diameter of 40–50 nm, mitochondria

and membrane elements of reticulum in the presynaptic axons terminals in intact muscle fibers. Several vesicles often form a group near the active zones of the presynaptic membrane. Axons located on the spicules of the sarcoplasm form zones of synaptic contacts (Fig. 3*A*) typical for locomotor muscles of insects. A similar picture was observed on days 4 and 8 after motor nerve cutting. The only difference was some decrease in the number of membrane elements of the reticulum. In individual cases consolidation of mitochondria was observed and sometimes hollow spots appeared in them (Fig. 3*B*, *C*). We never observed degenerative bodies such as these described in axons of leg muscles by other authors (Rees and Usherwood 1972). Interestingly beginning from day 4 after denervation synaptic vesicles began forming separate groups. A similar picture is observed in axons of denervated locust leg muscle (Rees and Usherwood 1972). Probably, accumulation of vesicles contributes to more massive release of transmitter and explains the appearance of "giant" MEPSP. On day 14 of denervation the vesicles formed large conglomerates which are at a distance from the presynaptic membrane and therefore do not take part in the processes of synaptic transmission. Moreover, there were no mitochondria in the axons (Fig. 3*D*). This morphological picture is observed when synaptic transmission has been blocked. Physiological experiments have shown that EPSP and MEPSP are absent at this stage.

On day 20 after denervation no axon profiles could be detected in muscle fibers. Probably, destruction of axon terminals occurred between days 14 and 20 after nerve cutting.

As mentioned above, Glu responses of denervated muscle fibers were characterized by a partial desensitization. This fact may be explained by the existence of two types of glutamate D-receptors on the muscle fiber membrane, one exposed to desensitization. It might be suggested that the receptors of this type are concentrated in the field of synaptic contacts and in intact muscles there are less accessible to applied glutamate. The nondesensitizating receptors are seemingly the extrasynaptic ones.

Our experiments confirmed that in the locust wing and leg muscles the motor nerve performs neurotrophic function and controls the distribution of Glu receptors in the membrane of muscle fibers. However, in contrast to leg muscles (Rees and Usherwood 1972) in neuromuscular synapses of wing muscle 120 no degenerative lamellar bodies were detected after denervation. The whole picture of postdenervation changes in the synapses of wing muscles was similar to that described for the locust leg muscles (Usherwood 1963 a,b; Rees and Usherwood 1972).

References

Alexandrov V. G., Baranov G. M. (1984): The effect of L-glutamic acid derivatives on neuromuscular synapses of the thergocoxal muscle of the locust *Locusta migratoria*. Zh. Evol. Biokhimii Fiziol. **20,** 422—425 (in Russian)

Gapon S. A., Kachman A. N., Frolova E. V. (1978): Studies of the chemoreceptive membrane in identified neuron of the gastropod *Planorbarius corneus* by different methods of drug application. Zh. Evol. Biokhimii Fiziol. **14,** 259—265 (in Russian)

Grigorev V. V. (1980): Physiological features of wing muscle fibre of the locust *Locusta migratoria migratorioides* R. F. Zh. Evol. Biokhimii Fiziol. **16,** 148—153 (in Russian)

Grigorev V. V., Mandelshtam Yu. E. (1981): Miniature potentials on fast and slow fibres of the locust. Neurofiziologiya **13,** 98—103 (in Russian)

Jan L. V., Jan Y. N. (1976):L-glutamate as an excitatory transmitter at the Drosophila larvae neuromuscular junction. J. Insect Physiol. **262,** 215—236

Mandelshtam Yu. E. (1983): Insect Neuron and Muscle, Nauka, Leningrad (in Russian)

Mathers D. A., Usherwood P. N. R. (1986): The sensitivity of the locust skeletal muscle fibres to L-glutamate following denervation and injury. Comp. Biochem. Physiol. **60** C, 7—10

Piek T. (1985): Neurotransmission and neuromodulation. In: Comprehensive Insect Physiology, Biochemistry and Pharmacology (Eds. G. A. Kerkut, L. J. Gilbert) pp. 55—118, Pergamon Press, Oxford

Rees D., Usherwood P. N. R. (1972): Fine structure of normal and denervated nerve muscle synapses in the locust *Schistocerca gregaria.* Comp. Biochem. Physiol. **43,** 83—101

Reanolds E. S. (1963): The use of the lead citrate of high pH as electron-opaque stain in electron microscopy. J. Cell. Biol. **17,** 208—212

Snodgrass R. E. (1935): Principles of Insect Morphology. McGraw-Hill, New York

Usherwood P. N. R. (1963a): Response of insect muscle to denervation. I. Resting potential changes. J. Insect Physiol. **9,** 247—255

Usherwood P. N. R. (1963b): Response of insect muscle to denervation. II. Changes in neuromuscular transmission. J. Insect Physiol. **9,** 811—847

Usherwood P. N. R. (1973): Release of transmitter from degenerating locust motoneurones. J. Exp. Biol. **59,** 1—16

Usherwood P. N. R. (1974): Nerve-muscle transmission. In: Insect Neurobiology, (Ed. J. E. Treherne) pp. 245—305, North Holland/Elsevier, Amsterdam, New York

Usherwood P. N. R., Machili P. (1968): Pharmacological properties of the excitatory neuromuscular synapses in the locust. J. Exp. Biol. **49,** 341—361

Final version accepted October 1, 1993