J. exp. Biol. **107**, 405–414 (1983)
Printed in Great Britain © *The Company of Biologists Limited 1983*

EFFECT OF LANTHANUM IONS ON NEUROMUSCULAR TRANSMISSION IN INSECTS

BY HIROSHI WASHIO AND TAKENORI MIYAMOTO

Laboratory of Neurophysiology, Mitsubishi-Kasei Institute of Life Sciences, Machida, Tokyo 194, Japan

(*Received 1 March 1983–Accepted 13 June 1983*)

SUMMARY

1. The effect of extracellular lanthanum on neuromuscular transmission was studied in cockroach leg muscle and larval mealworm ventral muscle by means of microelectrodes.

2. Miniature excitatory postsynaptic potential (MEPSP) frequency was markedly increased after lanthanum was added, in the presence and absence of calcium. The potentiation by La^{3+} was suppressed in a high Ca^{2+} saline and enhanced in the absence of Ca^{2+}.

3. Lanthanum ions blocked neuromuscular transmission at a concentration as low as 0·1 mM. The quantal content estimated by the failure method was reduced by 80 % in the presence of 0·1 mM-La^{3+}. The reduction in the EPSP amplitude by La^{3+} may be due to a decrease in the amount of transmitter released by a nerve impulse.

4. The response to L-glutamate applied iontophoretically was also reduced in the presence of La^{3+}. It seems unlikely that La^{3+} and L-glutamate were competing for a common binding site on the postsynaptic membrane since the apparent maximum of the dose-response curve for glutamate-induced depolarization was reduced in the presence of La^{3+}.

5. External recording of MEPSPs showed that adding lanthanum to the bathing medium increased the time constant of decay of the potential.

6. These results suggest that lanthanum does indeed have a postsynaptic action in addition to its prejunctional action in insect muscle fibres.

INTRODUCTION

Lanthanum produces blockage of neuromuscular transmission, perhaps due to a prevention of the influx of calcium ions into presynaptic nerve terminals (Miledi, 1966; Heuser & Miledi, 1971; Miledi, 1971). However, lanthanum also gives rise to a substantial increase in discharges of acetylcholine quanta (Blioch, Glagoleva, Liberman & Nenashev, 1968; Heuser & Miledi, 1971; DeBassio, Schnitzler & Parsons, 1972).

Recently, cobalt ions were found to have a dual effect on the spontaneous release of transmitter at insect motor nerve terminals, an inhibitory action being followed by an acceleratory action (Washio, 1982*a*). There was an antagonism by calcium of the inhibitory effect of cobalt. Since both cobalt (Weakly, 1973) and lanthanum (Blioch

Key words: Insect muscle, neuromuscular transmission, lanthanum ions.

et al. 1968; Heuser & Miledi, 1971; DeBassio *et al.* 1972) are more effective than magnesium in suppressing evoked transmitter release, we have studied the presynaptic effect of lanthanum at insect neuromuscular junctions. In this paper evidence is also presented to suggest that lanthanum has a postsynaptic action on the insect neuromuscular synapse.

MATERIALS AND METHODS

Experiments were performed on coxal depressor muscles of the cockroach, *Periplaneta americana*, muscle 178 from the metathoracic leg and 136 from the mesothoracic leg (Carbonell, 1947), and on the longitudinal ventral muscles of the larval mealworm, *Tenebrio molitor*. The standard bathing solution for cockroach muscles had the following composition (mM): NaCl, 158; KCl, 10·8; $CaCl_2$, 5; HEPES, 5; adjusted to pH 7·0 with NaOH. For mealworm muscles composition was NaCl, 70; KCl, 30; $CaCl_2$, 0·7; $MgCl_2$, 14·3; glucose, 445; HEPES, 5; adjusted to pH 7·2. Test solutions were prepared by substituting the appropriate cation chloride, for NaCl in the cockroach saline, and for glucose in the mealworm saline.

MEPSPs of cockroach muscle fibres were recorded intracellularly and extracellularly with micropipettes filled with 3 M-KCl and 2 M-NaCl, respectively. The EPSPs in *Tenebrio* muscle fibres were evoked by stimulating the segmental nerve innervating the muscle with supramaximal square pulses (0·01 ms) using a pair of silver hook electrodes. The iontophoretic electrodes were filled with 1 M-Na L-glutamate at pH 8·0 and had resistances of about 80 MΩ. The preparation was constantly perfused at a rate of 5·0 ml min^{-1}. Experiments were all performed at 20–24 °C.

RESULTS

Spontaneous release of transmitter

The experiments on spontaneous release of transmitter were performed on the cockroach muscle in order to compare with the effect of cobalt at the cockroach neuromuscular junction (Washio, 1982*a*), whereas the experiments on transmitter release by nerve impulses and on the response to L-glutamate applied iontophoretically were performed at the *Tenebrio* neuromuscular junction, since these mechanisms have been well documented in those muscles (Yamamoto & Washio, 1980).

In the presence of 5 mM-Ca^{2+}, increasing the concentration of La^{3+} caused an increase in frequency of MEPSPs recorded intracellularly. The higher the concentration, the faster the rate of rise of the frequency and the shorter the time lag between the application of the test solution and increase in the frequency (Fig. 1). Although the effect of external La^{3+} was almost the same in both normal and high K^+ (20·8 mM) saline, it seemed that the time lag and time course were shorter in high K^+ saline (Fig. 1). The high frequency caused by 5 mM-lanthanum lasted for less than 1 h at a K^+ concentration of either 10·8 or 20·8 mM (Fig. 1). Raising Ca^{2+} concentration while keeping La^{3+} concentration constant reduced the potentiation, but not below a control level (Fig. 2B). For example, when 0·1 mM-La^{3+} was introduced (at 0·1 mM-Ca^{2+} and 20·8 mM-K^+), it produced a sharp rise (more than one order of magnitude)

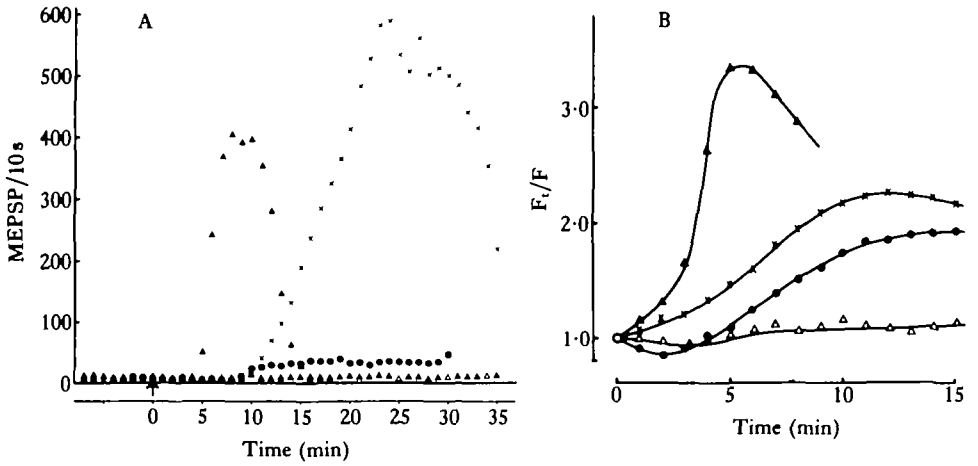

Fig. 1. Effect of elevating La^{3+} concentration on MEPSP frequency in saline containing 5 mM-Ca^{2+} on cockroach muscle fibres. (A) In a normal K^+ (10·8 mM) saline. (B) In a high K^+ (20·8 mM) saline. La^{3+} concentrations are 0·1 mM (△), 0·5 mM (●), 1·0 mM (×) and 5·0 mM (▲). The frequency on the ordinate in (B) is expressed as the ratio of the frequency in the test solution (F_t) to the frequency (F) in La^{3+}-free saline per 10 s. An arrow indicates the changeover point.

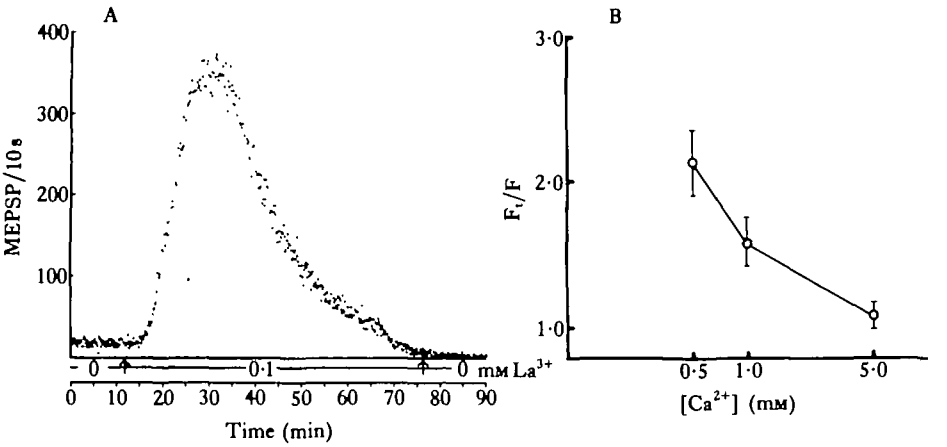

Fig. 2. (A) Time course of the effect of 0·1 mM-La^{3+} on the MEPSP frequency in cockroach muscle in a saline containing 0·1 mM-Ca^{2+}. (B) Effect of external calcium concentration on the potentiation of MEPSP frequency by 0·1 mM-La^{3+}. The frequency was measured 10–15 min after application of the La^{3+} saline. Arrows in (A) indicate the changeover points. Symbols on the ordinate in (B) as in Fig. 1B. Vertical bars indicate S.D. of each mean ($N = 5$). Both experiments were performed in high K^+ (20·8 mM) saline.

in the frequency of occurrence of MEPSPs after a lag of several minutes (Fig. 2A). After reaching a peak, MEPSP frequency declined to a very low level along a roughly exponential time course with a time constant of about 14 min in this fibre.

The time constant of the decay of the potentials recorded extracellularly depended on La^{3+} concentration. For example, in Fig. 3B, the time constant of the decay of the largest MEPSP was increased by 5 mM-La^{3+} from 0·61 ± 0·05 ms (mean ± S.D., $N = 12$) to 0·97 ± 0·18 ms ($N = 10$), ($P < 0·05$, Student's t-test). The frequency of

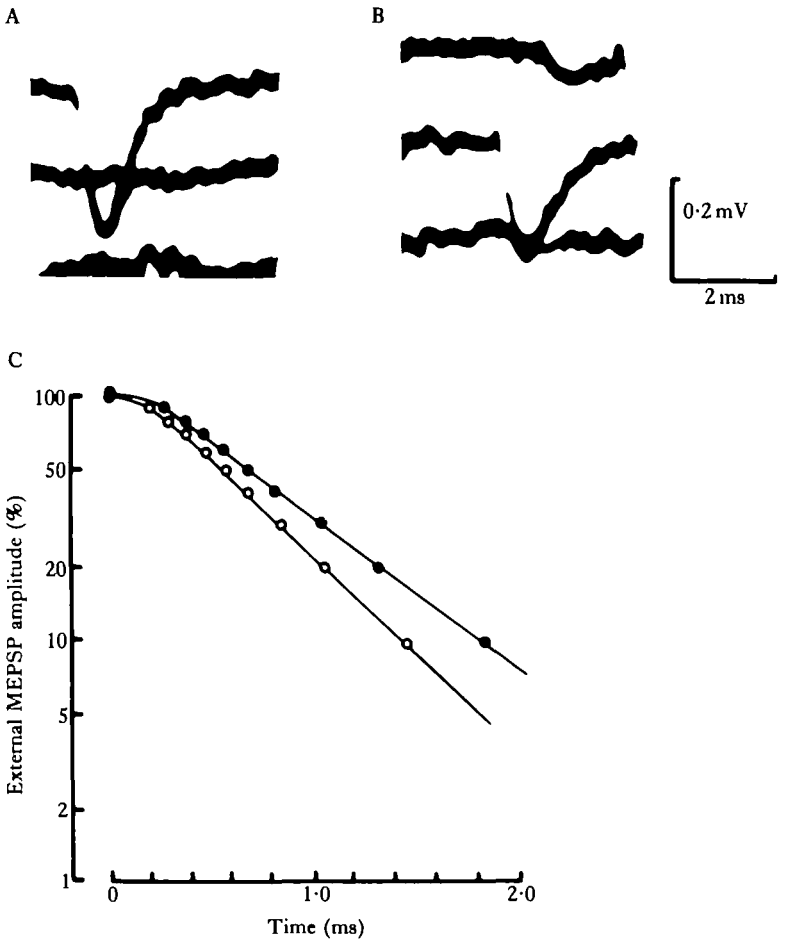

Fig. 3. Effect of 5 mM-La^{3+} on the time course of MEPSPs recorded extracellularly from a cockroach muscle fibre in saline containing 5 mM-Ca^{2+} and 20·8 mM-K^{+}. (A), (B) Examples of the MEPSP, control and 15 min after application of La^{3+} respectively. (C) Semilogarithmic plot of the decay of the potentials before (○) and 15 min after (●) application of 5 mM-La^{3+}. Time constants of the decay are 0·58 ms (○) and 0·72 ms (●).

MEPSPs recorded extracellularly was much lower than that recorded intracellularly in insect muscle fibres (Washio & Inouye, 1980).

Transmitter release by nerve impulses

As in frog muscle (Miledi, 1966; Heuser & Miledi, 1971), La^{3+} could not replace Ca^{2+} as a requirement for transmitter release in *Tenebrio* muscle. Release was not restored by 0·5 mM-La^{3+} after blockage in Ca-deficient medium.

Fig. 4A illustrates the reduction of the excitatory postsynaptic potential (EPSP) of the *Tenebrio* muscle fibre during the application of 0·5 mM-La^{3+}. The amplitude of the EPSP elicited by repetitive stimulation at 0·5 Hz was suppressed by 60% with perfusion for 2 min. The suppression of EPSP amplitude was almost irreversible (Fig

4A3). The relationship between the amplitude of EPSP and extracellular La^{3+} concentration, and the effect of La^{3+} on the time course of the EPSP are shown in Figs 5 and 6A, respectively. The reduction in the amplitude of EPSP produced by La^{3+} could be

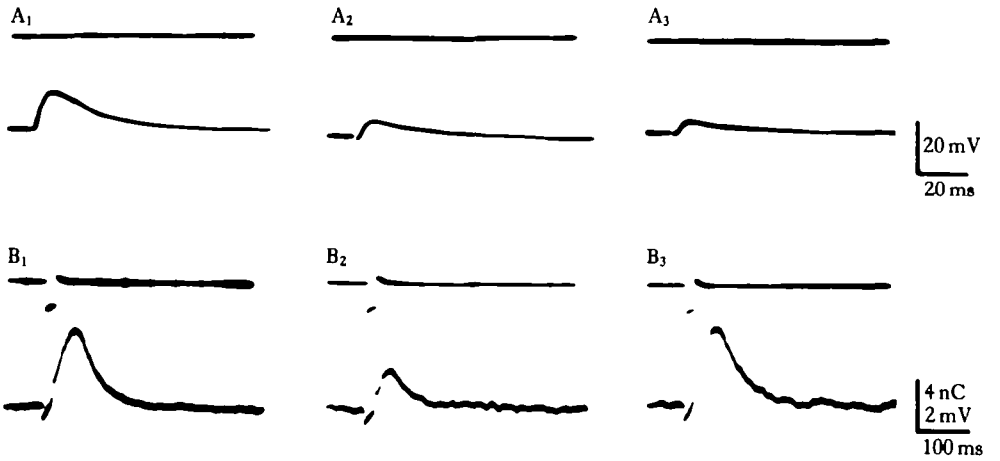

Fig. 4. Comparisons of the effect of 0·5 mM-La^{3+} on EPSP (A) and the response to the iontophoretically applied L-glutamate (glutamate potential, B). The EPSP and glutamate potential were simultaneously recorded from the same *Tenebrio* muscle fibre. A_1, B_1, before; A_2, B_2, 2 min after the application; A_3, B_3 7·5 min after washing out with La^{3+}-free saline containing 0·7 mM-Ca^{2+} and 14·3 mM-Mg^{2+}.

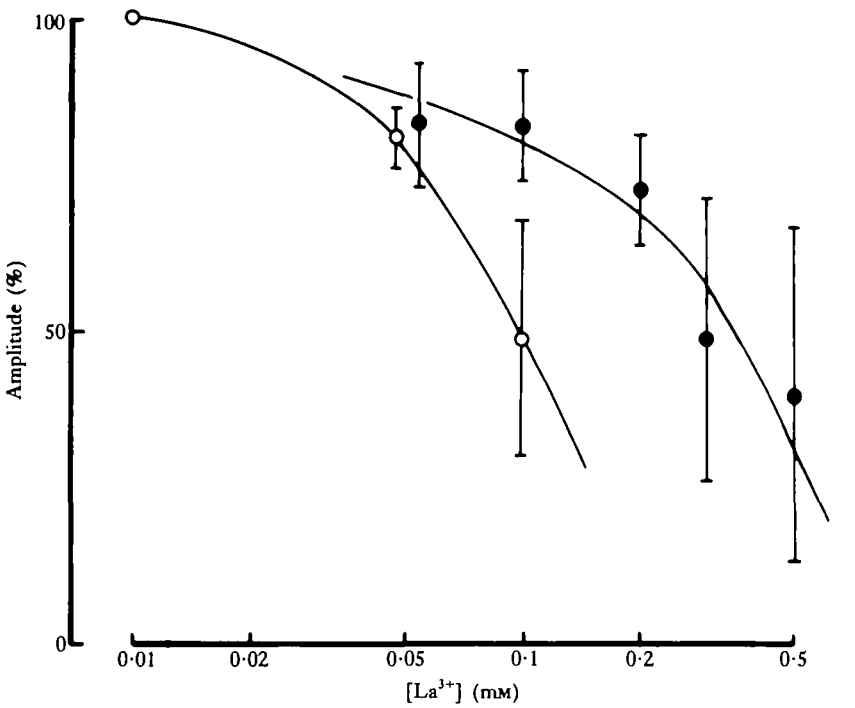

Fig. 5. Relation between EPSP amplitude (●), or the response to the iontophoretically applied L-glutamate (○), and extracellular La^{3+} concentration in a saline containing 0·7 mM-Ca^{2+} and 14·3 mM-Mg^{2+}. Amplitudes (mean ± S.D.) were measured at 10 min after application of La^{3+} to five *Tenebrio* muscle fibres.

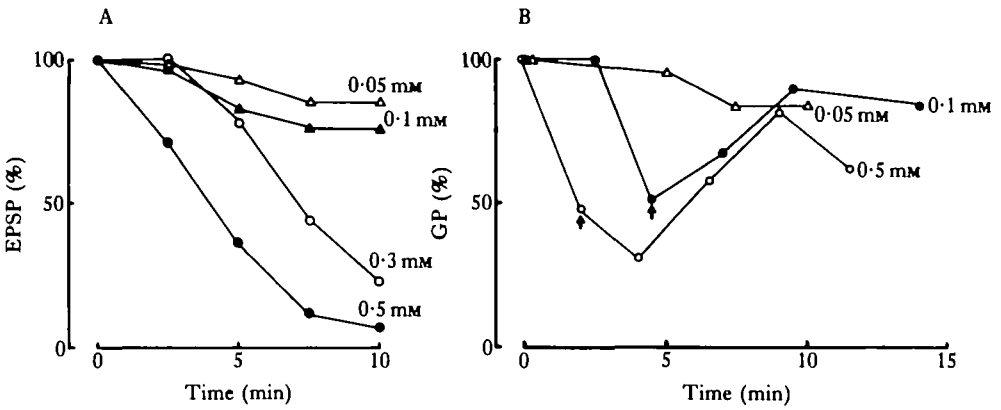

Fig. 6. Time course of the effect of La^{3+} concentration on EPSP amplitude (A) and on the glutamate potential (GP) amplitude (B) in saline containing 0·7 mM-Ca^{2+} and 14·3 mM-Mg^{2+} in *Tenebrio* muscle fibres. Ordinate represents percentage of the amplitude after the application of La^{3+} to the control. Arrows indicate washing out with a La^{3+}-free saline.

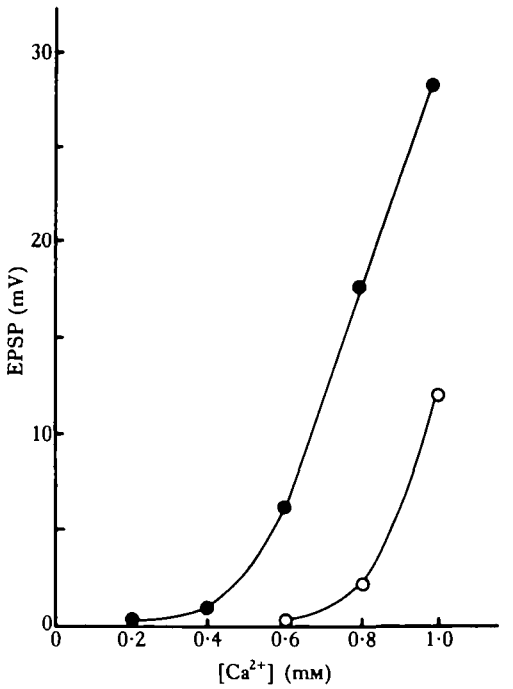

Fig. 7. Effect of external calcium concentration on the amplitude of EPSPs in the absence (●) and presence (○) of 0·1 mM-La^{3+} with 15 mM-Mg^{2+} in a *Tenebrio* muscle fibre.

antagonized to some degree by elevation of the external Ca^{2+} concentration (Fig. 7).

The quantal content was calculated from the number of failures of EPSP (del Castillo & Katz, 1954) in the absence and presence of La^{3+} at low Ca^{2+} concentration (0·2 mM-Ca^{2+} and 14·8 mM-Mg^{2+}) during repetitive nerve stimulation at 0·5 Hz (Table 1). The quantal content was reduced by 80 % in the presence of 0·1 mM-La^{3+}

Table 1. *Effect of lanthanum on quantal content (m)*

Fibre	Control (mc)	0·1 mM-La^{3+} (mt)	mt/mc
1	1·13	0·14	0·13
2	1·71	0·31	0·18
3	1·83	0·43	0·23
4	3·43	0·97	0·28
Mean and S.D.			0·20 ± 0·05

Effect of 0·1 mM-La^{3+} on the quantal content of *Tenebrio* EPSPs. The quantal content (m) was estimated from the ratio of total number of nerve stimulations to the number of failures of small EPSPs (<1 mV) equilibrated in a low Ca^{2+} (0·2 mM) and high Mg^{2+} (14·8 mM) saline. The number of stimuli ranged from 120 to 188. The stimulation rate was 0·5 Hz.

Thus, the reduction in the EPSP amplitude by La^{3+} may be due to a decrease in the amount of transmitter released by a nerve impulse.

The response to L-glutamate applied iontophoretically

Since glutamate is a putative transmitter in the *Tenebrio* muscle (Yamamoto & Washio, 1980), we studied the effect of La^{3+} on sensitivity to L-glutamate applied iontophoretically.

During the application of 0·5 mM-La^{3+}, the amplitude of the glutamate potential decreased by 50 % in 2 min after the application and recovered completely 10 min after washing with a La^{3+}-free saline (Fig. 4B). The time course of the recovery after washing was quite different from that of the neurally evoked response (cf. Fig. 4A and B). Also the threshold concentration of La^{3+} on the glutamate potential was found to be lower than that for the EPSP (Fig. 5). The difference of the threshold concentration and time course of the recovery between the response to L-glutamate applied iontophoretically and the EPSP may be attributed to the fact that the former is produced at a restricted junctional site while the latter is the integrated potential change of the junctions distributed over the whole muscle fibre.

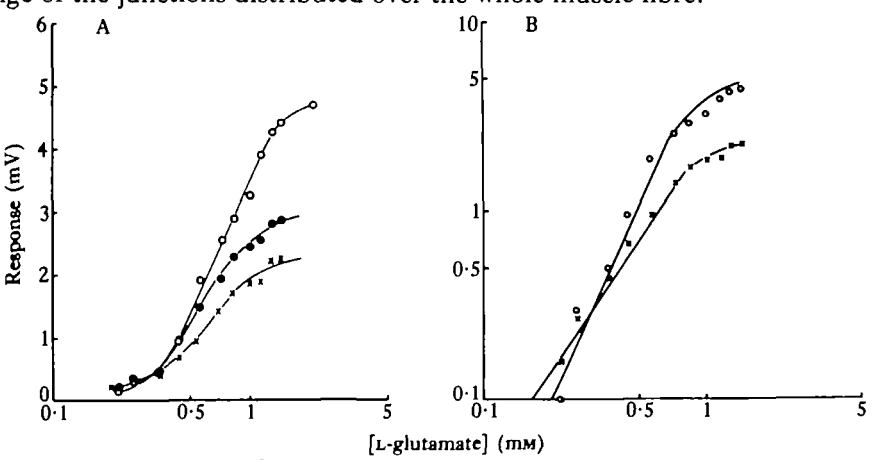

Fig. 8. Effect of 0·1 mM-La^{3+} on the dose-response curve to L-glutamate from a single *Tenebrio* muscle fibre. Semi- (A) and double (B) logarithmic plot of glutamate potential against the coulomb strength before (○), 10 min (●) and 15 min (×) after application of La^{3+} in the Ca^{2+}-free saline containing 15 mM-Mg^{2+}. The limiting slopes values in B are 2·6 (○) and 1·8 (×).

Lanthanum produced a reduction in the apparent maximum of the dose-response curve for glutamate-induced depolarization (Fig. 8). Double logarithmic plots of the amplitude of the depolarizing response to L-glutamate against coulomb strength of the ejection currents showed that the limiting slope values were 2·6 and 1·8 in the absence and presence of 0·1 mM-La^{3+} respectively (Fig. 8B). Thus, it seems unlikely that glutamate and lanthanum are competing for a common binding site on the postsynaptic membrane.

DISCUSSION

The present study in insect muscle fibres confirms the finding previously reported at frog neuromuscular junctions (Heuser & Miledi, 1971; DeBassio *et al.* 1972) that La^{3+} produces a substantial increase in spontaneous release of transmitter and blockage of neuromuscular transmission. Furthermore, our results indicate that La^{3+} brings about a reduction in the response to L-glutamate applied iontophoretically. The depression of the EPSP amplitude by La^{3+} could be accounted for by a decrease in the quantal content released by nerve impulses.

Although transmitter release by nerve impulses was decreased by extracellular La^{3+}, spontaneous release of transmitter was substantially increased. The potentiation of MEPSP frequency by La^{3+} was suppressed in a high Ca^{2+} saline and enhanced in the absence of Ca^{2+}. Thus, there might be an antagonism by Ca^{2+} of the La^{3+} acceleration of spontaneous release. DeBassio *et al.* (1972) have reported an antagonism between them and suggested that the competition might occur at anionic binding sites on the external surface of the nerve terminal. An inhibitory action on the spontaneous release of transmitter which has been found in the presence of Ca^{2+} (Washio, 1982*a*) has not been clear in the presence of La^{3+}. The increase in the frequency was recorded in the absence of external Ca^{2+} or the presence of a very low concentration. Therefore, it seems more likely that La^{3+} acts on an intracellular site (DeBassio *et al.* 1972; Washio, 1982*b*). For example, La^{3+} has been reported to be an extremely potent inhibitor of the energy-linked accumulation of Ca^{2+} by mitochondria (Mela, 1968, 1969) and thereby increases the intracellular calcium concentration (Rahamimoff, 1978). However, MEPSP frequency was found to be lowered slightly in the presence of 5 mM-Ca^{2+} just after the application of 0·5 mM-La^{3+} in some cockroach muscle fibres partially depolarized with high K^+ (Fig. 1B). It might be possible that an inhibitory effect of La^{3+} on the external surface of the nerve terminal was followed by the acceleratory effect which masked the former.

Our results also showed that lanthanum ions decreased the amplitude of the transient depolarization (glutamate potential) produced by iontophoretically applied L-glutamate. Double logarithmic plots of the amplitude of the depolarizing response to L-glutamate against coulomb strength of the ejection currents showed that La^{3+} reduced the value for the slope of the junctional receptors. Therefore, it seems unlikely that L-glutamate and La^{3+} are competing for a common binding site on the postsynaptic membrane. Alternatively, La^{3+} might affect the kinetics of the channels activated by L-glutamate, since the trivalent ion can modify the time course of the MEPSP recorded extracellularly. It has been shown that the kinetics of L-glutamate-induced channels may be altered by the species of permeable cations (Cull-Candy &

Miledi, 1982) as well as the kinetics of acetylcholine-induced ones (Gage & Van Helden, 1979; Bregestovski, Miledi & Parker, 1979; Magleby & Weinstock, 1980). On the other hand, it has been proposed that the change in the decay time of the end-plate current can be explained by screening fixed negative charges on the end-plate membrane (Mallart & Molgó, 1978; Cohen & Van der Kloot, 1978). The kinetics of the channels depend upon the voltage gradient through the postsynaptic membrane. In insect muscles, hyperpolarization shortens the decay time of MEPSCs and depolarization lengthens the decay time (Cull-Candy & Miledi, 1982) which is in the opposite direction to the kinetics in vertebrate muscles (Anderson, Cull-Candy & Miledi, 1978) and to the behaviour expected from screening fixed negative charges (Cohen & Van der Kloot, 1978). Thus, the results obtained in insect muscles are incompatible with the surface charge interpretation. Recently, (+)-tubocurarine-induced block of the glutamate synapse resulting from plugging the open channels was reported in *Tenebrio* muscle fibres (Yamamoto & Washio, 1983). Thus, it might be possible that La^{3+} can alter the kinetics of glutamate channels by acting on channel properties as a non-competitive antagonist, like a local anaesthetic, or by changing the rates with which L-glutamate interacts with receptors, like acetylcholine and strontium (Miledi & Parker, 1980).

The authors wish to thank Professor David M. J. Quastel, Department of Pharmacology, University of British Columbia, for his helpful comments on the manuscript. Thanks are also due to Miss Junko Kaneko for her technical assistance.

REFERENCES

ANDERSON, C. R., CULL-CANDY, S. G. & MILEDI, R. (1978). Glutamate current noise: post-synaptic channel kinetics investigated under voltage clamp. *J. Physiol., Lond.* **282**, 219–242.

BLIOCH, Z. L., GLAGOLEVA, I. M., LIBERMAN, E. A. & NENASHEV, V. A. (1968). A study of the mechanism of quantal transmitter release at a chemical synapse. *J. Physiol., Lond.* **109**, 11–35.

BREGESTOVSKI, P. D., MILEDI, R. & PARKER, I. (1979). Calcium conductance of acetylcholine-induced end-plate channels. *Nature, Lond.* **279**, 638–639.

CARBONELL, C. S. (1947). The thoracic muscles of the cockroach. *Periplaneta americana. Smithson. misc. Collns* **107**(2), 1–23.

COHEN, I. & VAN DER KLOOT, W. (1978). Effects of $[Ca^{2+}]$ and $[Mg^{2+}]$ on the decay of miniature end-plate currents. *Nature, Lond.* **271**, 77–79.

CULL-CANDY, S. G. & MILEDI, R. (1982). Properties of miniature excitatory junctional currents at the locust nerve-muscle junction. *J. Physiol., Lond.* **326**, 527–551.

DEBASSIO, W. A., SCHNITZLER, R. M. & PARSONS, R. L. (1972). Influence of lanthanum on transmitter release at the neuromuscular junction. *J. Neurobiol.* **2**, 263–278.

DEL CASTILLO, J. & KATZ, B. (1954). Quantal components of the end-plate potential. *J. Physiol., Lond.* **124**, 560–573.

GAGE, P. W. & VAN HELDEN, D. (1979). Effects of permanent monovalent cation on end-plate channels. *J. Physiol., Lond.* **288**, 509–528.

HEUSER, J. & MILEDI, R. (1971). Effect of lanthanum ions on function and structure of frog neuromuscular junctions. *Proc. R. Soc.* B**179**, 247–260.

MAGLEBY, K. L. & WEINSTOCK, M. M. (1980). Nickel and calcium ions modify the characteristics of the acetylcholine receptor-channel complex at the frog neuromuscular junction. *J. Physiol., Lond.* **299**, 203–218.

MALLART, A. & MOLGÓ, J. (1978). The effects of pH and curare on the time course of end-plate currents at the neuromuscular junction of the frog. *J. Physiol., Lond.* **276**, 343–352.

MELA, L. (1968). Interaction of La^{3+} and local anesthetic drugs with mitochondrial Ca^{2+} and Mg^{2+} uptake. *Archs Biochem. Biophys.* **123**, 286–293.

MELA, L. (1969). Inhibition and activation of calcium transport in mitochondria, effect of lanthanides and local anesthetic drugs. *Biochemistry, N.Y.* **8**, 2481–2456.

MILEDI, R. (1966). Strontium as a substitute for calcium in the process of transmitter release at the neuromuscular junction. *Nature, Lond.* **212**, 1233–1234.

MILEDI, R. (1971). Lanthanum ions abolish the 'calcium response' of nerve terminals. *Nature, Lond.* **229**, 410–411.

MILEDI, R. & PARKER, I. (1980). Effect of strontium ions on end-plate channel properties. *J. Physiol., Lond.* **306**, 567–577.

RAHAMIMOFF, R. (1978). Intracellular and extracellular calcium ions in transmitter release at the neuromuscular synapse. *Ann. N.Y. Acad. Sci.* **307**, 583–598.

WASHIO, H. (1982*a*). A dual effect of cobalt ions on the spontaneous release of transmitter at insect motor nerve terminals. *J. exp. Biol.* **98**, 353–361.

WASHIO, H. (1982*b*). Effect of uncoupling agents of oxidative phosphorylation on the spontaneous release of transmitter from insect motor nerve terminals. *Comp. Biochem. Physiol.* **72**C, 369–373.

WASHIO, H. M. & INOUYE, S. T. (1978). The effect of calcium and magnesium on the spontaneous release of transmitter at insect motor nerve terminals. *J. exp. Biol.* **75**, 101–112.

WASHIO, H. M. & INOUYE, S. T. (1980). The statistical analysis of spontaneous transmitter release at individual junction on cockroach muscle. *J. exp. Biol.* **87**, 195–201.

WEAKLY, J. N. (1973). The action of cobalt ions on neuromuscular transmission in the frog. *J. Physiol., Lond.* **234**, 597–612.

YAMAMOTO, D. & WASHIO, H. (1980). L-glutamate as an excitatory transmitter at the neuromuscular junction of a beetle larva. *J. Insect Physiol.* **26**, 253–256.

YAMAMOTO, D. & WASHIO, H. (1983). Voltage jump analysis of curare action at a glutamate synapse. *J. Neurophysiol.* **49**, 396–405.