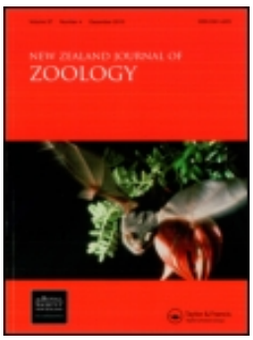

New Zealand Journal of Zoology

ISSN: 0301-4223 (Print) 1175-8821 (Online) Journal homepage: http://www.tandfonline.com/loi/tnzz20

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To cite this article: C. E. Franklin & L. H. Field (1985) Effect of venom from Latrodectus katipo and Ixeuticus martius (Arachnida: Araneae) on insect neuromuscular transmission, New Zealand Journal of Zoology, 12:2, 175-180, DOI: 10.1080/03014223.1985.10428277

To link to this article: http://dx.doi.org/10.1080/03014223.1985.10428277

Published online: 30 Jan 2012.

New Zealand Journal of Zoology, 1985, Vol. 12: 175–180
0301-4223/85/1202-0175$2.50/0

Effect of venom from *Latrodectus katipo* and *Ixeuticus martius* (Arachnida: Araneae) on insect neuromuscular transmission

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Abstract Homogenates of spider venom glands were applied topically to fibres from the extensor tibiae muscles of adult male American cockroaches (*Periplaneta americana*) from which miniature end plate potentials (MEPPs) were recorded with an intracellular glass capillary microelectrode. Venom from *Latrodectus katipo* caused a sudden, transitory increase in MEPP frequency, which peaked and then decreased over the next hour until no MEPPs were recorded. Small, random fluctuations (3–5 mV) in the resting potential of the muscle fibres were also recorded. *Ixeuticus martius* venom caused a rapid, logarithmic decrease in MEPP frequency; no MEPPs were recorded 15–20 min after venom application. The resting potential decreased by 30–40 mV 5–10 min after application. *L. katipo* venom appears to act presynaptically, causing a massive release of transmitter. Although a presynaptic mechanism for *I. martius* venom has not been excluded, it is more likely that the observed effect results from a post-synaptic blockade of transmitter receptor sites.

Keywords spiders; *Latrodectus katipo*; *Ixeuticus martius*; venom; Theridiidae; neurophysiology; neuromuscular junctions; *Periplaneta americana*

INTRODUCTION

The spiders are one of the largest groups of venomous animals: except for the Uloboridae and Heptathelidae, which have lost their venom glands secondarily (Bettini & Brignoli 1978), all spiders are venomous. Most research on spider venoms has concentrated on the few species of medical importance: the black widow spider (*Latrodectus mactans*); the Australian funnel web spider (*Atrax robustus*); the brown recluse spider (*Loxosceles reclusa*); and wolf spiders of the genus *Lycosa*. The biological role of venoms in the vast majority of spiders is unknown.

The katipo spider, *Latrodectus katipo* Powell, 1871 (Theridiidae), is known in New Zealand as a potentially dangerous spider; it belongs to a cosmopolitan genus which includes such well known species as the Australian red back *L. hasselti*, and the American black widow *L. mactans*. *Ixeuticus martius* Simon, 1899 (= *Badumna longinquus* and *Amaurobius longinquus*) is probably the commonest spider in New Zealand (R. R. Jackson pers. comm.), but its venom is not known to be medically important, nor has it been physiologically characterised. This paper describes the effects of venom from these 2 species on miniature end plate potentials (MEPPs) at insect neuromuscular junctions and on resting membrane potentials recorded from insect muscle fibres. We present additional information on the venom of the genus *Latrodectus*, and new information on the venom of a poorly known genus, *Ixeuticus*.

MATERIALS AND METHODS

Spiders were collected from coastal dunes (*L. katipo*) or urban dwellings (*I. martius*) near Christchurch, New Zealand, and kept individually in plastic vials for 3 days to ensure that their venom sacs were full before being frozen in liquid nitrogen and stored in a freezer for later use. After thawing, the venom glands of *L. katipo* were removed by pulling gently on the chelicerae. With *I. martius*, the removal of the venom glands was more difficult. The anterior half of the cephalothorax was dissected dorsally and the glands, which are just underneath the cuticle, were gently pulled free by fine forceps. The venom glands of either species were homogenised in a measured volume of ice-cold cockroach saline in a glass tissue-homogeniser. The supernatant of the venom gland homogenate was applied directly to the neuromuscular preparations. The concentrations of venom gland homogenates used were 6 glands per 250 µL for *L. katipo*; and 4 glands per 500 µL for *I. martius*.

The neuromuscular preparation consisted of semi-exposed metathoracic extensor tibiae muscles of adult male American cockroaches (*Periplaneta*

Received 1 October 1984; accepted 16 November 1984

americana). An isolated metathoracic leg severed above the coxa was positioned firmly in sticky wax and a small opening exposing the fibres of the extensor tibiae muscle was made in the cuticle. The muscle was constantly bathed in cockroach saline (Becht et al. 1960).

Intracellular recordings of MEPPs and membrane potentials were made with glass microelectrodes filled with potassium acetate; their resistances were 10–30 MΩ. Muscles fibres were successively probed with the microelectrode until a good, stable recording of MEPPs was achieved. The microelectrode was positioned primarily in the surface muscle fibres.

To test the effects of venom, the pool of saline surrounding the microelectrode was withdrawn and replaced with 5 μL of the standard homogenate of venom glands. The amplitude and the frequency of MEPPs were monitored and recorded at regular intervals to obtain the time course of venom action. Two control solutions were used: a muscle homogenate (obtained from spider cheliceral muscles and homogenised in cockroach saline) was applied to the preparation and left for 1 h; and the cockroach saline without venom was tested for a 2 h period.

RESULTS

Effect of venom on MEPPs

Spontaneous MEPPs varied in amplitiude between 0.05 and 0.8 mV, with durations of 10–15 ms. Both Fig. 1A and 1B show the waveform and time course of MEPPs recorded from a normal, quiescent preparation. The unitary nature of these potentials is demonstrated as well as the summation of close MEPPs (Fig. 1A). Although these recordings are unusually clear, we recognise that a limitation of this preparation is the polysynaptic innervation of the muscle fibres; the records may not necessarily demonstrate the activity of a single synapse.

Fig. 2A shows an example of the effect of *L. katipo* venom on MEPPs following application of venom homogenate to the extensor tibiae muscle. The sweep speed in these records is much slower than those in Fig. 1, hence the MEPPs appear compressed. The time course of *L. katipo* venom action on MEPPs is plotted in Fig. 3. The large standard error bars reflect the differences in the MEPP frequency in 4 preparations. Shortly after katipo venom was applied to the neuromuscular preparation the frequency of spontaneously occurring MEPPs increased sharply, and reached a maximum of 12–24× the normal frequency in 30 min. The MEPP frequency decreased rapidly after the peak (to 50% of maximum frequency at 42 min), and no MEPPs were recorded 80 min after the

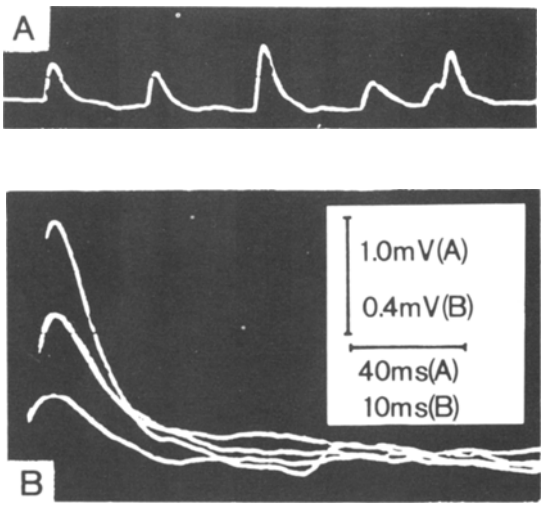

Fig. 1 Miniature end plate potentials recorded from cockroach extensor tibiae muscle before spider venom was applied.

venom was applied. The effect of the katipo venom could not be reversed by intermittent washing with physiological saline for 30 min. Both the time and height of the peak MEPP frequency varied considerably from preparation to preparation and the results of 16 preparations are summarised in Table 1.

Frequency distributions of MEPP amplitudes were recorded during the course of venom action. At the higher MEPP frequencies, single MEPPs were superimposed on each other, which caused an increase in MEPP amplitude; this made accurate counting of MEPPs very difficult and probably resulted in a conservative estimate of the frequency of MEPPs. Before the venom was applied, most of the MEPPs recorded had an amplitude of less than 0.2 mV (Fig. 4A). Twenty min after application of venom, the amplitudes of the MEPPs increased. More MEPPs in the range 0.1 to 0.2 mV were recorded (Fig. 4B). Thirty min after the venom was applied the MEPP amplitudes had increased even further, with a few MEPPs having amplitudes greater than 0.4 mV (Fig. 4C). After 60 min, most MEPPS had amplitudes of less than 0.1 mV (Fig. 4D).

In all of the preparations mentioned, venom gland homogenates from adult females were used. When venom gland homogenates from adult male katipos were applied to the extensor tibia muscle preparation, the MEPP frequency increased and then decreased to zero, as for venom from females.

The venom gland homogenates of *I. martius* had a completely different effect upon the frequency and amplitude of spontaneous MEPPs in cockroach

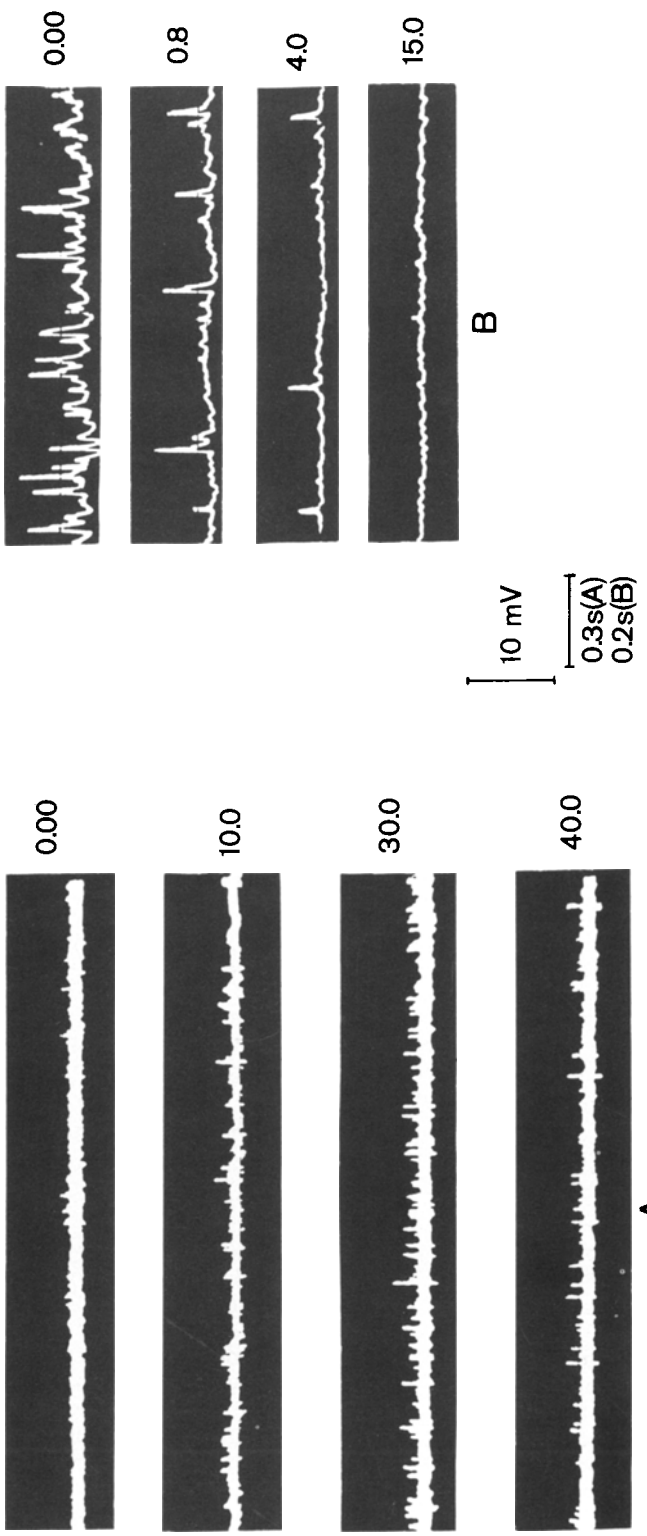

Fig. 2 Action of spider venoms on cockroach extensor tibiae muscle. (A) the effect of *Latrodectus katipo* venom applied at time = 0.00. Note the increase in both the frequency and amplitude of MEPPs by 30 min (time indicated on right side), then the subsequent decline. (B) The effect of *Ixeuticus martius* venom. The initial MEPP frequency and amplitude declined rapidly until the MEPPs disappeared by 15 min. Note the differences in sweep speed in (A) and (B).

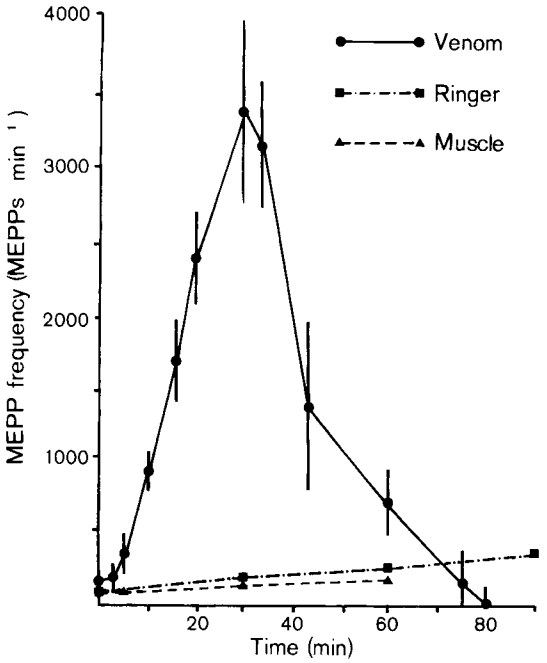

Fig. 3 Effect of *Latrodectus katipo* venom and control solutions on MEPP frequency in cockroach extensor tibiae muscle (mean ± SE, n=4).

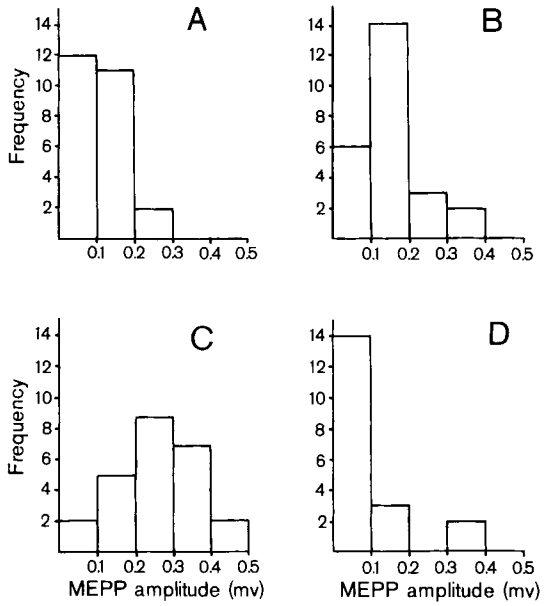

Fig. 4 Frequency distribution of MEPP amplitudes before and after application of *Latrodectus katipo* venom. (A) Before venom applied. (B), (C), (D): 20, 30, and 60 min after venom applied, respectively.

muscle. Fig. 2B shows the effect on MEPPs of applying *I. martius* venom to the extensor tibiae muscle; the time course of venom action averaged from 4 preparations is shown in Fig. 5. The venom caused a rapid decrease in the MEPP frequency and amplitude within 10 s of application. The frequency decreased logarithmically (Fig. 5) and reached 0 after 15–20 min. The rapid initial decrease in MEPP frequency after application of the venom slowed after 2–3 min, and no MEPPs were recorded after 15 min. Intermittent washing with physiological saline for 30 min did not reverse the venom action. The MEPPs did not reappear during 30–60 min after their disappearance.

As with katipo venom, there was much variation between different preparations when *I. martius* venom was applied. The MEPPs disappeared after 4–19 min (mean 11.4 min n=11).

Neither the cockroach saline nor the muscle homogenate (Fig. 3) significantly changed the MEPP frequency, especially in comparison to the effect of the venoms. After 60 min in the cockroach saline the baseline frequency had increased by 100%, in the muscle homogenate the frequency had increased by 24%.

Effect of venom on resting potentials

Katipo venom caused small, random fluctuations (3–5 mV) in the resting potential of the muscle fibres. These were observed shortly after the venom was applied and also towards the final stages of the venom's action. When *I. martius* venom was applied, the resting potential remained relatively stable for 2–3 min, but after 5 min the resting potential slowly decreased from 60–80 mV to reach 30–40 mV at 20–25 min, for the sample of muscle fibres tested.

DISCUSSION

The venom of *L. katipo* appeared to affect the presynaptic membrane of insect neuromuscular junctions by causing an increase in the release of chemical transmitter (glutamic acid) and hence an increase in the MEPP frequency. After 40–120 min, the venom had permanently exhausted the release of transmitter and its action, and this could not be reversed by washing with physiological saline. Similar results were obtained by Griffiths & Smyth (1973) when they applied homogenate of the venom

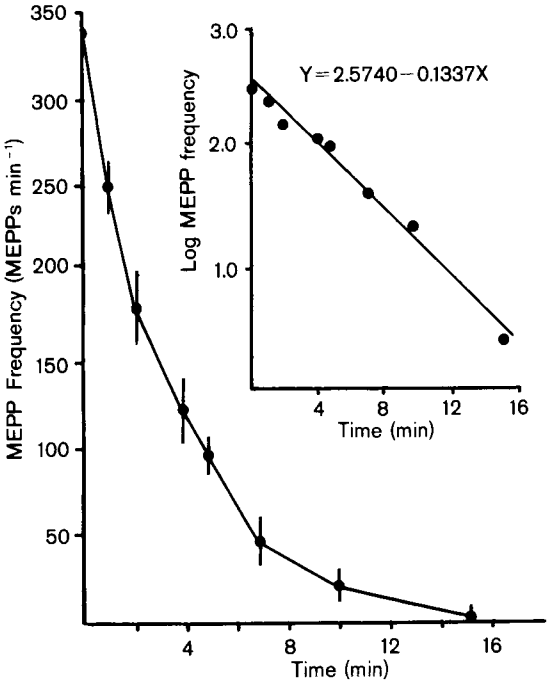

Fig. 5 Effect of *Ixeuticus martius* venom on MEPP frequency in cockroach extensor tibiae muscle (mean ± SE). (Inset, semi-logarithmic transformation of same data, showing a highly significant linear relationship $r=0.994$, $P<0.01$.

Table 1 Summary of effects of *Latrodectus katipo* venom on MEPP in cockroach muscle, ($n=16$; frequencies in MEPPs min^{-1}; time in minutes.)

Parameter	Mean	Range
Control frequency	321	63–600
Time to peak frequency	30	15–45
Maximum frequency	2651	1515–3660
Time until zero MEPPs	75	40–120

glands of *L. mactans* to the metathoracic flexor tibiae muscles of the American cockroach. They reported a massive spontaneous release of transmitter progressing to electrical silence and permanent synaptic blockage in both excitatory and inhibitory neuromuscular junctions. Cull-Candy et al. (1973) applied *L. mactans* venom to the metathoracic extensor tibiae and retractor unguis muscle fibres of the locust *Schistocerca gregaria*, and also observed an increase in MEPP frequency, although the increase occurred in irregular bursts. Thus the venoms of *L. katipo* and *L. mactans* appear to be similar in their effect upon transmitter release.

The venom of *L. mactans* has been studied intensively. It has been applied to the cholinergic neuromuscular junctions of the frog (Longenecker et al. 1970; Clark et al. 1970) with results essentially the same as those for insect glutaminergic synapses. Clark et al. (1972) investigated the effect of *L. mactans* venom on the fine structure of the neuromuscular junctions of the frog and found that the number of synaptic vesicles in the nerve terminal was reduced. The presynaptic membrane of the nerve terminal had infolded or had lifted.

The mechanism by which *Latrodectus* venom induces a massive quantal release of transmitter is not known with certainty (Howard & Gundersen 1980). Venom from the black widow spider has been shown by Finkelstein et al. (1976) to increase the cation conductance of artifical lipid bilayer membranes, which suggests that the venom can insert itself into the lipid bilayer. Gorio et al. (1978) found that the same venom acted on the plasma membrane of the nerve terminal by increasing its permeability to Ca and Na ions and this may stimulate transmitter release. The venom also stimulates transmitter release by an unknown mechanism, which does not depend on these changes in permeability. Baba & Cooper (1980) postulated that *Latrodectus* venom binds to either glycoproteins or gangliosides or both on the synaptic membrane, thereby opening a channel which allows the translocation of monovalent cations. The subsequent depolarisation of the synaptosomes would inhibit uptake processes and permit the movement of transmitters along their concentration gradients, independent of the Ca-linked excitation-secretion coupling.

Male *Latrodectus* are generally considered to be harmless and their venom has not been studied. That they are harmless is related to the morphology of the chelicerae and not to their having a less potent venom. Male katipos are only half the size of a mature female, and their fangs are so small that they are unable to penetrate human skin (unpublished data). The size of the venom glands is also a factor in determining the lethality of the bite.

It is difficult to determine whether *I. martius* venom has a presynaptic or postsynaptic effect upon the neuromuscular junctions of the cockroach. The decrease in MEPP amplitude suggests that the venom affects the postsynaptic membrane. This is supported by the decrease in muscle fibre resting potential that occurs 5–10 min after the venom is applied. The venom may also act presynaptically, as the rapid decrease in MEPP frequency occurred before the muscle resting potential had decreased substantially.

Abe et al. (1983) found that application of the venom of *Nephila clavata* (Araneidae) to the neuromuscular synapse of a lobster decreased the amplitude of the excitatory postsynaptic potentials, but it did not affect the inhibitory postsynaptic potentials. The venom was shown to block specifically the glutamate receptors in the lobster neuromuscular junction. *Ixeuticus martius* and *N. clavata* venoms seem to act similarly, despite the 2 species not being closely related phylogenetically. The action of *N. clavata* venom had a very short time course in which the amplitude of the excitatory postsynaptic potentials decreased exponentially to less than 10% within 15 min. One action of *I. martius* venom could be to block receptors in the postsynaptic membranes of insect neuromuscular junctions. The venom could act to open K or Ca membrane channels rapidly, which would explain the rapid and irreversible depolarisation of the muscle which was observed. Further work will be needed to test these assumptions.

ACKNOWLEDGMENTS

We thank Dr R. R. Jackson and Susan Hallas for advice and comments.

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