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The Effect of *Habrobracon* Venom on Excitatory Neuromuscular Transmission in Insects*

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Summary. 1. The action of the venom of the braconid wasp *Habrobracon hebetor* was studied in nerve-muscle preparations of its host the mealmoth larva (*Ephestia kühniella*) and of the locust (*Locusta migratoria*) by comparing physiological properties of normal and paralysed preparations.

2. The venom neither had an effect on nervous conduction nor on membrane potential, current-voltage relationship, graded electrogenesis and contraction of the muscle fibres.

3. Inhibitory transmission was not blocked. Excitatory junction potentials were either greatly diminished or absent during paralysis. They could not be restored to normal size by increasing the external Ca concentration.

4. The postjunctional sensitivity to L-glutamic acid was not significantly altered by the venom.

5. In paralysed locust preparations miniature excitatory junction potentials were still observed, but their frequency was reduced to as little as 1% of controls. Their amplitude distributions were similar to controls, except that the proportion of very large mejps was somewhat higher.

6. In weakly paralysed preparations tetanic nerve stimulation caused facilitation and posttetanic potentiation of the reduced ejps. With more extensive paralysis, tetanic stimulation increased the frequency of the mejps and a few of the stimuli were followed by mejp-like ejps.

7. Raising the osmolarity of the saline increased the mejp-frequency of paralysed preparations significantly less than in control preparations.

8. The thiol oxidizing compound diamide caused a large increase of mejp-frequency in controls but completely blocked spontaneous release in paralysed preparations. These effects could be quickly reversed by a subsequent application of the disulfide reducing agent dithiothreitol.

9. It is unlikely that the purely presynaptic effect of *Habrobracon* venom is on the electrical properties of the excitatory nerve terminals. It is discussed whether the release mechanism or the supply of transmitter are affected. There may be a specific affinity of the venom to glutaminergic synapses.

Introduction

The females of certain hymenopterous insects paralyse other insects or spiders by injecting venom through their sting. The venom of the

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diggerwasp *Philanthus* for example does primarily not affect the central nervous system but acts peripherally (Rathmayer, 1962a, b) by blocking neuromuscular transmission through a presynaptic effect on both excitatory and inhibitory junctions (Piek *et al.*, 1971).

The action of the venom of the braconid wasp *Habrobracon (Microbracon) hebetor* on Lepidoptera was studied by several authors (Beard, 1952; Piek, 1966; Rathmayer, 1966; Piek and Engels, 1969; Piek and Mantel, 1971). The venom also acts peripherally by blocking neuromuscular transmission, apparently on the presynaptic side, but only affects excitatory junctions.

In view of this specificity, and because of the possible usefulness of *Habrobracon* venom as a pharmacological tool for synaptic research in arthropods, it seemed desirable to investigate its action in more detail. For the present study two animals were chosen: larvae of the mealmoth *Ephestia*, which are the natural prey of *Habrobracon*, and locusts, because most of the current knowledge on neuromuscular transmission in insects has been obtained from this animal.

Material and Methods

The general approach was to examine the physiological properties of nerve-muscle preparations from already paralysed insects. This has the advantage that experiments can be carried out under well controlled conditions. Such experiments are free of possible side effects which have to be taken into account when for example gland extracts are applied to preparations in vitro.

Preparations. The larvae of the mealmoth (*Ephestia kühniella*) were cut open dorsally and pinned out in a small perspex chamber. Gut and salivary glands were removed. The exposed longitudinal muscles of the ventral body wall were studied. These could be stimulated indirectly with the aid of a suction electrode attached to a connective of the ventral nerve cord.

In the locust (*Locusta migratoria*) either the retractor unguis preparation (Usherwood and Machili, 1968) or more often the extensor tibiae of the hindleg were used. In the latter muscle some of the ventral fibres on the distal end are innervated by an inhibitory axon which could be selectively stimulated by an axon reflex (Usherwood and Grundfest, 1965). To compare the properties of paralysed muscles with those of control muscles control experiments were done, usually first, with the unparalysed contralateral hindleg from the same animal. To record mejps sometimes 4th instar larvae were used because of a better signal to noise ratio.

Venom Application. The *Habrobracon* strain we used was originally supplied by Dr. Drescher (University of Bonn) and has been reared for several years in our laboratory using mealmoth larvae as hosts. The species was kindly determined by Dr. J. Papp, Budapest, as *Habrobracon hebetor* Say.

With *Ephestia* larvae were used which had been stung by a wasp and which were totally immobilized by the venom. No differences were noted between the physiological properties of preparations investigated from one hour up to two days after the onset of paralysis.

With the locust a small volume of saline (5 µl) containing the venom was injected into a hindleg one to two days prior to the experiment. For this purpose a small hole was cut in the cuticle of the femur. When this operation was made on control animals and locust saline injected there was no effect on neuromuscular transmission.

Venom was prepared in one of two ways. Two or three wasps were forced to eject venom into a small glass capillary over a 30 min period. The dried secretion, which could be stored at −5 to −10°C for months, appeared to be almost insoluble in locust saline yet released toxin into it. Alternatively venom glands from frozen wasps were homogenized in saline (usually 25 into 10 µl) and centrifuged. The supernatant was taken into capillaries and was either used immediately or deep frozen until used within a few days. No qualitative difference was noted between the effect of secreted venom and gland extract but injection of an amount of gland extract equivalent to the contents of about 3 glands (which was the standard procedure) in general acted more quickly and led to a stronger paralysis than did secreted venom.

Solutions and Drugs. For *Ephestia* the following saline was used: Na^+ 32.6, K^+ 32.7, Mg^{++} 25.5, Ca^{++} 20.6, Cl^- 157.5 (mM/l) according to Duchateau *et al.* (1953), with 250 mM/l sucrose added. The locust saline contained: Na^+ 154, K^+ 10, Ca^{++} 2, and Cl^- 168 (mM/l). The solutions were buffered with 0.2 M tris maleate to pH 7.4 with *Ephestia* and 6.8 with the locust respectively. The bath was perfused continuously except for periods of 5 to 10 minutes when drugs were applied. In order to check glutamate sensitivity a rapid constant saline flow (approx. 20 bath volumes/min) was used. Glutamate saline was applied several times and each time was washed out for at least 2 minutes. Its concentration (9×10^{-6} to 4×10^{-5} M/l) was adjusted to cause a peak depolarization of approximately 5 mV.

Diamide, kindly supplied by Dr. E. M. Kosower, Tel Aviv University, Israel, and dithiothreitol (A-grade, Calbiochem) were dissolved in locust saline and ejected onto the muscle directly from a small pipette.

Electrophysiological methods for recording and stimulating were conventional. For recording mejps a low pass filter with cut-off frequency of 1 KHz was used.

Results

General Observations

Once *Ephestia* larvae have been stung by *Habrobracon* they become immobilized within a few minutes. Although completely flaccid they stay alive for many days. Recovery was never observed.

After injection of venom into a jumping leg of a locust it takes at least 15 min before the first signs of paralysis can be detected. One to several hours later the femoro-tibial part of this leg is completely immobilized. Squeezing the tarsus of the paralysed leg still causes reflex movements in other legs indicating that nervous conduction in afferent fibers is not blocked. Depending on the dose of venom injected, paralysis either spreads over the whole animal or recovery sets in very slowly. In the latter case even one month later venom-treated legs appear to be weaker and are unable to perform a jumping movement.

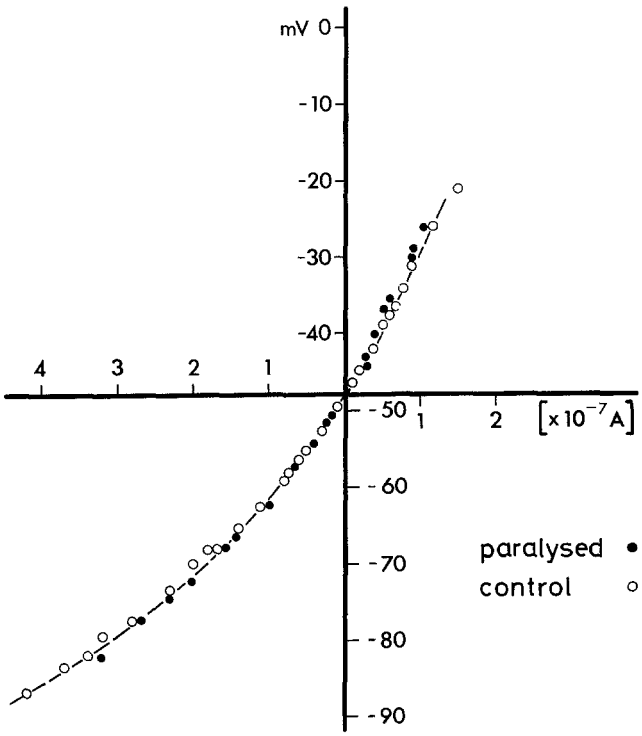

Fig. 1. Current-voltage relationship of a muscle fibre paralysed for two days and a control fibre of *Ephestia*. There is no difference in input resistance and anomalous rectification. In both preparations the resting membrane potential was −48 mV

Nervous Conduction, Electrical Parameters of the Muscle Membrane, and Excitation-Contraction Coupling

The venom did not affect the conduction in peripheral nerves. Even in completely paralysed locust legs action potentials of normal size could be recorded extracellularly from motor nerves. The conduction velocity (between 2 and 4 m/sec) of an identified motor axon ranged from 80 to 120% of that in controls from unparalysed contralateral legs of the same animal.

Neither in *Ephestia* nor in *Locusta* was there any statistically significant effect of the venom on the resting membrane potential of the muscle fibres.

The current-voltage relationship of *Ephestia* muscle fibres is also not affected by the venom (Fig. 1). Furthermore, in paralysed retractor unguis fibres from locusts, input resistances did not significantly differ from control values. In both muscle preparations depolarizing current pulses

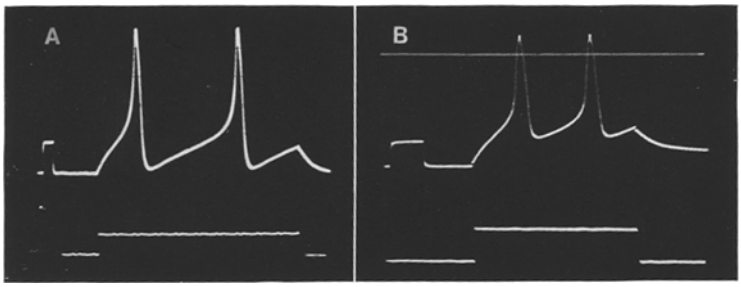

Fig. 2. Electrically excited membrane response in muscle fibres of *Ephestia*. Direct stimulation with depolarizing current pulses of 1.4×10^{-7}. Amp. A Normal preparation. B Paralysed for 15 hrs. The upper line represents zero potential. Calibration pulses 10 mV, 10 msec

of sufficient strength caused graded electrogenesis up to an overshooting spike-like response in paralysed as in control muscles (Fig. 2). In both cases these membrane responses were followed by twitch-like contractions of the muscle fibre.

The observations demonstrate that the electrical properties of the non-synaptic muscle membrane are not altered and that excitation-contraction coupling is not blocked by *Habrobracon* venom.

Excitatory and Inhibitory Junction Potentials

Stimulation of the connectives in a paralysed mealmoth preparation does not result in any sign of postsynaptic response in the ventral body wall muscles. In control preparations the response consists of a large

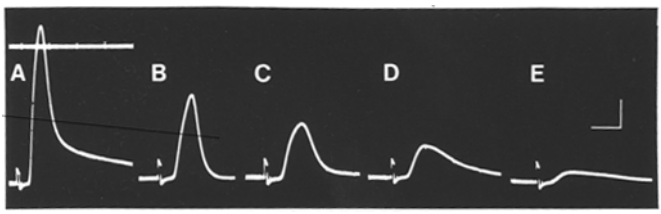

Fig. 3. Changes in postsynaptic responses of *Ephestia* muscle on application of *Habrobracon* venom. A The normal response consists of a large ejp leading to an electrically excited response overshooting zero potential (upper line). B—E Gradual decline of the response; recordings taken 3.5, 4, 4.5, 5 and 6 min respectively after venom application to the bath. In D and E only the ejp is left. Calibration bars: 10 mV, 5 msec

Fig. 4. Excitatory junction potentials in paralysed locust retractor unguis muscles. A Weak paralysis; the ejp resembles an ejp recorded from a normal preparation in the presence of a high Mg concentration. B Stronger paralysis; three successive sweeps superimposed. C Normal response in an undepressed control preparation consisting of a large ejp which elicits an electrically excited membrane response. Calibration bars: Vertical, 1 mV in A, 0.5 mV in B, 10 mV in C. Horizontal, 20 msec in A and B, 40 msec in C

excitatory junction potential (ejp) leading to an overshooting electrically excited response (Fig. 3 A). Fig. 3 B to E shows the gradual decline of this response on application of venom to the preparation in vitro.

In muscles from completely paralysed locust legs, stimulation of the nerves usually does not evoke ejps. Tetanic stimulation even at high frequencies is not effective in causing contraction. In some cases, however, probably reflecting a low dose of injected venom, reduced or sometimes very small erratic ejps (Fig. 4A, B) were recorded from retractor unguis muscle fibres.

In contrast to the results with ejps, inhibitory junction potentials (ijps) could be found in all paralysed extensor tibiae preparations (Fig. 5). With reversal potentials below −60 mV the ijp-amplitudes ranged from 1 to 5 mV if the resting membrane potentials were around −50 mV. This agrees well with the values from control preparations. In *Ephestia* inhibitory innervation of the ventral body wall muscles is apparently lacking like in the waxmoth larva (Belton, 1969).

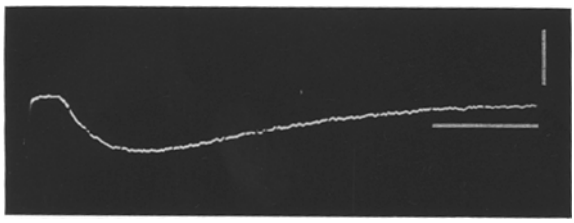

Fig. 5. Inhibitory junction potential in a paralysed locust extensor tibiae muscle. Calibration bars: Vertical, 2 mV; horizontal, 20 msec. Resting membrane potential −48 mV. Citrate electrode

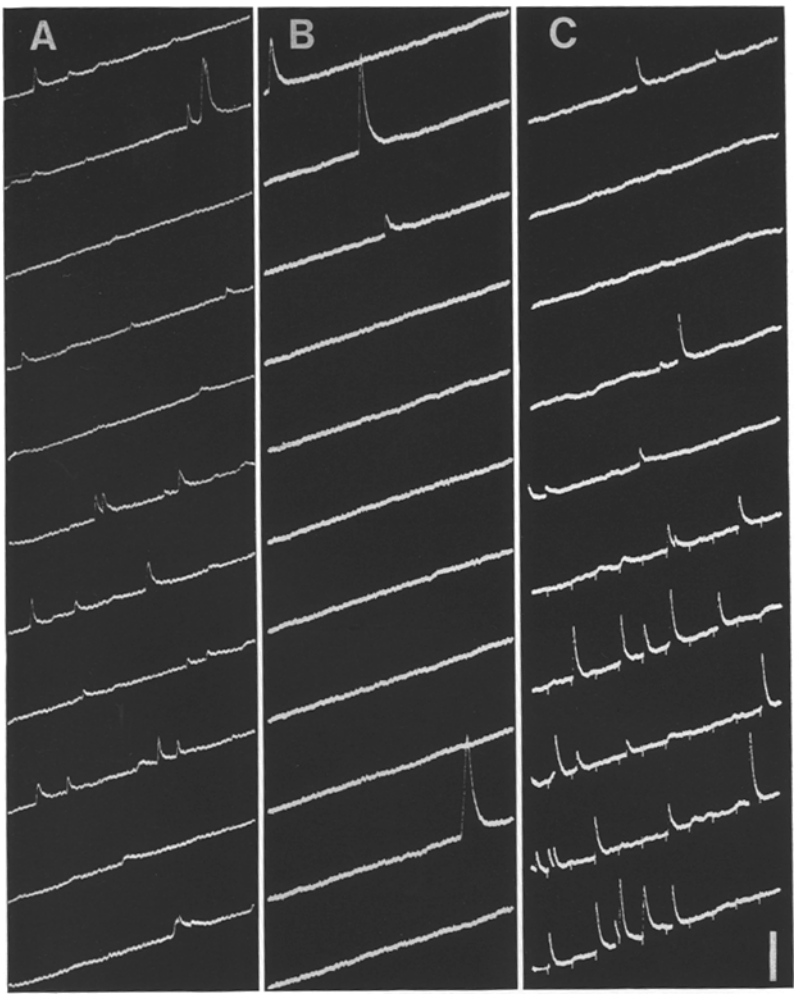

Fig. 6. Miniature excitatory junction potentials in normal and paralysed locust extensor tibiae muscle. A Control. B and C Paralysed. In C repetitive nerve stimulation in the five lower sweeps causes ejps and extra mejps. Calibration bar: 1 mV in A and B, 2 mV in C. Sweep duration 1.1 sec in A and B, 2.2 sec in C. Records from 4th instar larvae. Stimulus artifacts retouched

Frequency and Amplitude of Miniature Excitatory Junction Potentials

In paralysed locust muscles miniature excitatory junction potentials (mejps) still occur, apparantly at random, but at a lower frequency (Fig. 6).

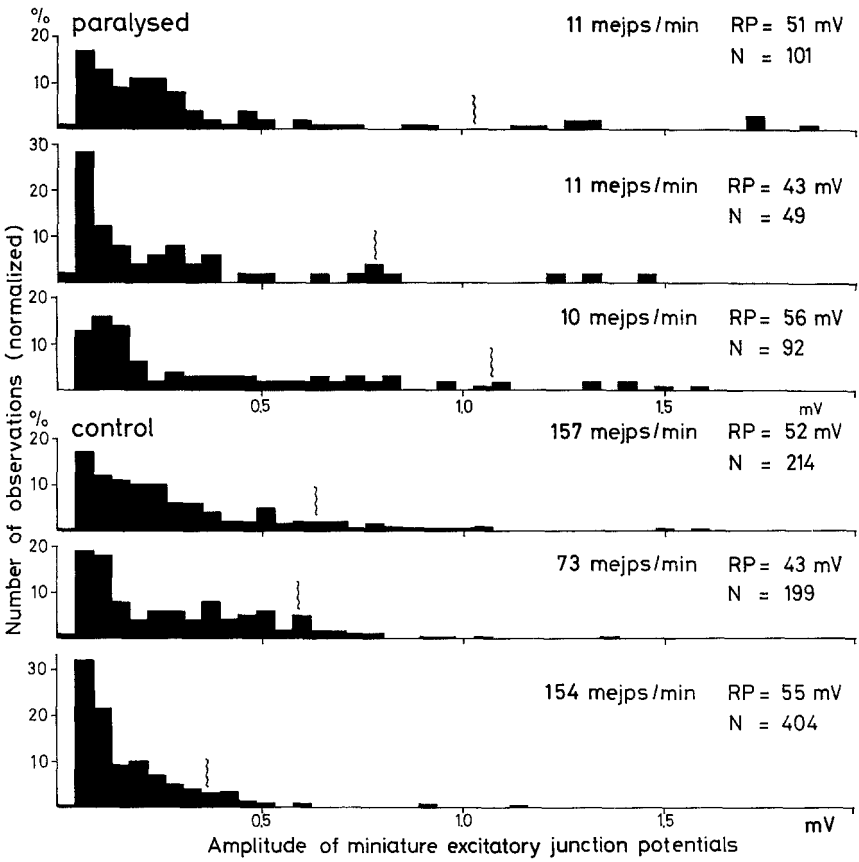

Fig. 7. Amplitude histograms of mejps from locust extensor tibiae muscle. Upper part: Paralysed hindleg. Lower part: Control of contralateral hindleg of the same animal. RP, resting membrane potential; N, number of observations. For each graph the corresponding mejp-frequencies are indicated. Data from a 4th instar larva

Whereas in normal preparations the frequency is around 300/min (range 80–600/min), it is decreased to 3–10/min one day after standard application of venom (cf. Methods). The amount by which the frequency has dropped reflects the degree of intoxication. Thus in a case where small ejps could still be evoked the frequency of the mejps was between 50 and 60/min whereas in a preparation from a heavily intoxicated animal the intervals between successive mejps were often longer than one minute.

Amplitude histograms of mejps recorded from paralysed preparations are similar to those from controls (Fig. 7) but the proportion of very large mejps is greater (statistically significant; χ^2-test, $p<0.001$). These

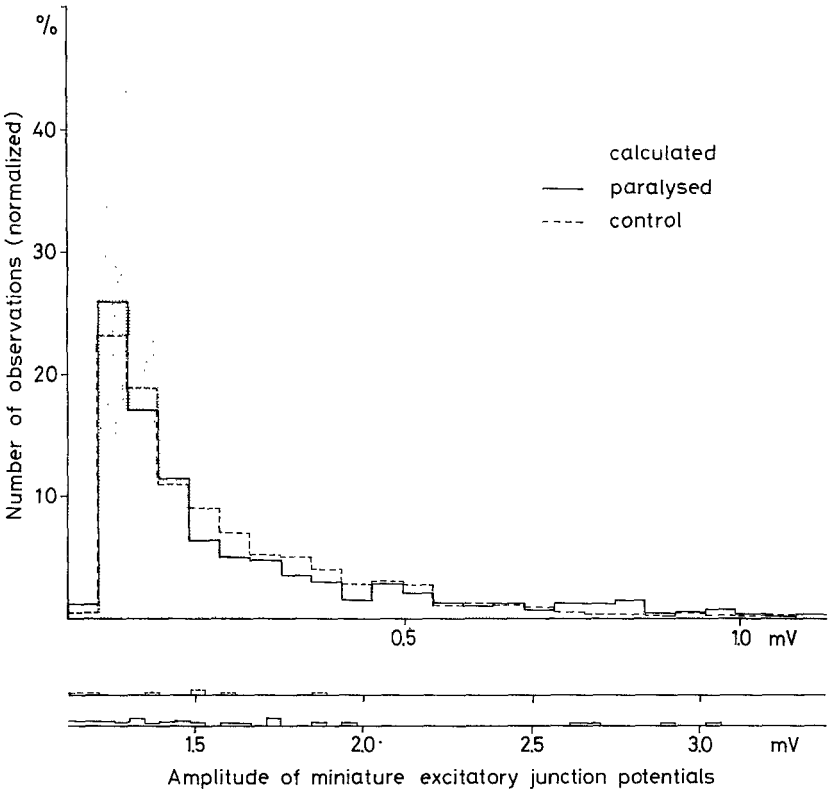

Fig. 8. Amplitude histograms of mejps from locust extensor tibiae muscle. Averaged distributions from six recordings from normal and seven recordings from paralysed preparations. For reasons of clarity the continuation of the x-axis is shown separately for the data of paralysed and unparalysed preparations in the lower part of the graph. Note difference in scale. The shaded histogram represents the calculated amplitude distribution of the control preparation assuming a general reduction of the mejp-amplitudes to $^1/_3$ and excluding the class of smallest amplitudes, which would be obscured by the noise. Data from two 4th instar larvae

very large mejps have the same time-course and duration as the smaller ones. Usually the largest amplitudes reach higher values than in control preparations. For the class of mejps with very low amplitudes distributions skewed as in Fig. 7 may be misleading since one has to assume that the smallest mejps are hidden in the noise.The question arises as to whether all or at least part of the observed reduction in mejp-frequency could have resulted from a reduction of the mean amplitude of the mejps. This could be due either to a reduction of quantum size or to a reduced postsynaptic efficiency of the transmitter. In either case, the very large

mejps in paralysed preparations would have to be considered to represent a different category, e.g. potentials caused by a number of quanta released synchronously.

Fig. 8 shows the kind of amplitude distribution which would be expected in paralysed preparations if the average mejp-amplitude were reduced to 1/3 of its original value. The difference between this calculated distribution and that observed in paralysed preparations is significant ($p<0.0005$; χ^2-test). It would become still greater if a reduction to less than 2/3 was assumed. With a reduction of the mejp-amplitude by less than 1/2 on the other hand the calculated and the observed distribution cannot be distinguished safely any more.

If we consider the mejp-frequencies under the above assumptions, drops to 47% or 77% are expected for amplitude reductions to $^1/_3$ or $^1/_2$ respectively. Since, however, a decrease to 15% was observed with the paralysed preparations in our case (Fig. 8) one has to conclude that it represents mainly if not exclusively a direct effect of the venom on transmitter release. Should there be, in addition, some reduction of the mejp-amplitude, it could not be greater than 50%. Such a reduction, if due to a decreased transmitter sensitivity of the postjunctional membrane should become evident in the following experiments.

Postjunctional Sensitivity to Glutamate

L-glutamate is very likely to be the excitatory transmitter in crustacean and insect skeletal muscles (for review see Gerschenfeld, 1973). If the postjunctional sensitivity to transmitter were reduced by the venom, one would expect a reduced response to applied glutamate. Testing glutamate sensitivity is also an additional means for deciding whether the main action of the venom is on the pre- or postsynaptic side.

The experiments with glutamate were performed on the retractor unguis muscle of paralysed and normal hindlegs of locusts. In both preparations glutamate (approx. 10^{-5} M/l; applied to the bath) caused depolarizations of around 5 mV (cf. Methods). Their time course was similar and there was no statistically significant difference in the peak depolarizations. Thus the venom has no postsynaptic effect at all.

Transmitter Release under Various Conditions

a) Increase in Ca-Concentration. Raising the external Ca-concentration from 2 to 10 mM/l did not cause the return of normal transmission in paralysed locust leg muscles. In weakly paralysed preparations, where ejps of 1 mV could still be evoked by single nerve stimuli, there was an increase of the amplitudes by a factor of 3. Part of this increase may be due to an increase in membrane resistance of the muscle fiber.

b) Tetanic Nerve Stimulation. In weakly paralysed preparations, where single nerve stimuli evoked small ejps (Fig. 4), facilitation and post-tetanic potentiation still occurred with repetitive stimulation. With higher degrees of paralysis, where the mejp-frequency was not yet drastically decreased, but 1 Hz stimulation no longer evoked ejps, tetanic stimulation with 20 Hz or more usually caused ejps to reappear. Their amplitudes ranged up to a few mV. Upon returning to 1 Hz stimulation, the amplitude of these ejps were further potentiated for a short period of time, then decreased, and eventually the ejps disappeared.

With mejp-frequencies as low as 1–3/min, indicating a deep paralysis, only part of the stimuli during tetanic stimulation evoked ejps with amplitudes similar to those of mejps (Fig. 6C). In addition, the mejp frequency was increased during the period of stimulation, as has been shown also for normal preparations (Usherwood, 1972). The sum of the numbers of ejps and mejps during a stimulus train lasting a few seconds increased with the stimulus frequency. For frequencies of 10 to 20 Hz the sum ranged between 2 and 50 times the number of mejps observed in an equal interval without stimulation. With long stimulus trains, however, this increased release was maintained only during the first 5 to 10 seconds.

In the vertebrate endplate which is blocked by botulinum toxin (Spitzer, 1972) or by tetanus toxin (Duchen and Tonge, 1973), it was shown that on tetanic stimulation quanta of increased average size were released. Although the effect of *Habrobracon* venom resembles the action of these two toxins no convincing evidence for a similar effect was seen in our experiments. This question however was not investigated in full detail.

c) Raised Osmolarity. If locust saline containing 500 mM/l sucrose was put on a normal extensor tibiae preparation the mejp-frequency invariably rose within a few minutes by a factor of 10 to 15. In paralysed preparations, where the mejp-frequency was depressed to 5 to 20% of the controls, the same treatment hardly caused an increase (by less than a factor of 2).

d) Action of Diamide and Dithiothreitol. The thiol oxidizing agent diamide has been shown to greatly enhance the frequency of mejps in the frog motor end-plate (Werman *et al.*, 1971). This was found to be also true in the normal locust leg muscle preparation: after application of 7.5 mM/l diamide the frequency of mejps went up by a factor of 8 to 15 within 5 minutes even if ejps had been completely blocked by the presence of 40 mM/l Mg in Ca-"free" saline. Diamide by itself did not block ejps at least within 15 minutes after it was applied. If the drug was washed out after 5 minutes the mejp-frequency remained increased for at least 30 minutes.

Table 1. Effect of diamide and dithiothreitol on the frequency of the mejps

	Sequence of drug application		
	diamide →	washing →	dithiothreitol
mejp-frequency in			
a) normal	increase	maintained increase	return to initial
b) paralysed preparations	drop to zero	zero	return to initial

Dithiothreitol (DTT) which reduces disulphide linkages (Cleland, 1964) has at a concentration of 1 mM/l hardly any effect on neurally evoked contractions in the grasshopper *Romalea* (Bratkowski *et al.*, 1972).

In the present experiments, application of DTT (10 mM/l) to a normal extensor tibiae did not significantly affect the mejp-frequency within 10 minutes. After washing out DTT, application of diamide had the usual effect on mejp-frequency. If, however, diamide was first put on for 5 minutes, then was washed off for another 5 minutes and finally DTT was added, the mejp-frequency returned to about the initial value within 2 to 3 minutes.

In paralysed preparations (mejp-frequencies reduced to 5–15/min) the effects of diamide and DTT treatment were opposite to what was found in the controls: 2 to 5 minutes after application of diamide mejps disappeared. They did not reappear after washing the preparation within 20 minutes. Tetanic nerve stimulation in this situation was ineffective.

If DTT was applied 5 to 10 minutes after washing off the diamide, the mejps appeared within a few minutes. With a reversed sequence of drug administration, DTT neither increased the mejp-frequency nor did it prevent the effect of the subsequent diamide treatment. The effects of diamide and DTT on the frequency of the mejps are summarized in Table 1. Both drugs had little effect on the amplitudes of the mejps and on resting membrane potentials in normal and paralysed muscles, and there was no significant change of membrane input resistance.

Discussion

It has previously been thought that *Habrobracon* venom was active only in lepidopterous insects and possibly a few species of Diptera and Hemiptera (Waller, 1965a; Drenth, 1969; Piek and Simon Thomas, 1969). That the venom in fact can also paralyse locusts might be due to a difference in dose of venom applied. Furthermore, the toxicity of venom from different strains of *Habrobracon hebetor* can apparently differ by two orders of magnitude (Piek, pers. communication).

In neuromuscular preparations of both the mealmoth and the locust there was no indication for any postsynaptic effect of the venom. This as well as the finding that inhibitory neuromuscular transmission was not blocked agrees with the observations for the moth *Philosamia* (Piek and Engels, 1969; Piek and Mantel, 1970). Although the present data on mejp-amplitudes and mejp-frequencies do by themselves not fully rule out the possibility that there might be some reduction of postjunctional transmitter sensitivity, this seems unlikely because no change in sensitivity to glutamate was found.

It is also unlikely that the venom prevents the nerve action potentials from invading the nerve terminals at excitatory junctions: Nervous conduction in excitatory motor axons appears to be normal, and since inhibitory transmission is not blocked one would have to assume that there are specific differences between the properties of electrically excitable membranes of excitatory and inhibitory axons. Even in deeply paralysed preparations tetanic nerve stimulation still increased excitatory transmitter release. This indicates that conduction of electrical signals into the excitatory nerve terminal is not blocked completely if it is reduced at all.

There remain two possible presynaptic modes of action of the venom: The pool from which transmitter is drawn during release could be depleted, or the release mechanism itself could be affected. These two possibilities are not mutually exclusive.

An effect of the venom on the release mechanism could have a similar basis as the effect of high concentrations of external Mg, which is thought to compete with Ca for a common site and thus interferes with excitation-secretion coupling (Rubin, 1970). There are four kinds of evidence which indicate that the venom does not act in this way: a) The frequency of the mejps was reduced to a much higher degree than is generally found for high Mg or low Ca concentrations (Hubbard, 1970; Usherwood, 1963). b) Raising the external Ca concentration does not restore transmission to normal. c) Increased tonicity of the saline does not markedly increase the mejp-frequency as it does in normal preparations; this increase, at least in the frog motor endplate (Blioch *et al.*, 1968), is not prevented by high Mg. d) Application of diamide stops spontaneous release in paralysed preparations, whereas it increases mejp-frequency in normal preparations even when transmission is blocked by high Mg and low Ca. It should be kept in mind, however, that there could be alternative possibilities by which the venom affects transmitter release.

The other possibility of a presynaptic mode of action of the venom is a depletion of the transmitter pool. Although compatible with this idea, our present data do not yet provide specific support for it. A depletion of the pool by black widow spider venom (BWSV) has been demonstrated in a variety of synapses (Longenecker *et al.*, 1970; Clark

et al., 1972; Frontali, 1972; Kawai *et al.*, 1972; Cull-Candy *et al.*, 1973). The action of *Habrobracon* venom is different from that of BWSV in that it causes no dramatic transient increase in mejp-frequency (Piek and Engels, 1969; Piek and Mantel, 1970; Walther, unpublished). Furthermore, after two days of paralysis, neuromuscular junctions are still full of synaptic vesicles (electronmicroscopical observations on the ventral body wall muscles of *Ephestia* and on the retractor unguis of the locust; Rathmayer and Walther, unpublished).

If the venom severely slows down the supply of transmitter quanta to be released, then the pool might become depleted almost completely by the ongoing release. The supply mechanism would then become rate limiting for the release. Factors which greatly enhance release under normal conditions should be either ineffective during paralysis or cause a smaller increase not only in absolute but also in relative terms.

The finding that the antagonism between diamide and dithiothreitol, although still present during paralysis, is of opposite sign, does at least not contradict the assumption of a pool depletion. This reversal raises the question of whether sulfhydryl-groups or disulphide bonds play a role in the mechanism of venom action. For the black widow spider venom, for example, it has been demonstrated that the toxic effect can be prevented if cysteine is added to the venom prior to application (Chmouliovsky *et al.*, 1972). With *Habrobracon* venom, however, no inactivation was achieved by cysteine or DTT (Walther, unpublished). Nor was paralysis prevented by 5,5′-dithiobis-(2-nitrobenzoic acid), which quantitatively oxidizes sulfhydryl-groups (Ellman, 1959).

One particularly interesting property of *Habrobracon* venom is that it may specifically act on glutaminergic synapses. The following two points lend support to this hypothesis: a) Inhibitory neuromuscular transmission in the locust where GABA is thought to be the transmitter (Usherwood and Grundfest, 1965), is not blocked by the venom. b) Cholinergic transmission at the frog motor endplate is not blocked by *Habrobracon* venom (Deitmer *et al.*, 1974).

That in *Ephestia* excitatory neuromuscular transmission is mediated also by glutamate is likely from the observation that application of glutamate (10^{-3} M/l) blocks ejps reversibly by desensitization of the postjunctional receptors (Rathmayer, unpublished). Preliminary experiments on the blocking effect of *Habrobracon* venom in crustacean neuromuscular junctions give further evidence bearing on the assumed specificity of this venom for glutaminergic synapses (Deitmer *et al.*, 1974).

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