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Philanthotoxin: A New Model for an Additional Class of Insecticides

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Abstract. Philanthotoxin is an extract from the venom sac of the solitary digger wasp *Philanthus triangulum* F. The venom is active in all insects and the toxic component is a peptide polyamine. The effects of the venom on cholinergic and glutamatergic synapses were studied using electrophysiological techniques. *Philanthus* toxin at a very low concentration (0.04 unit/ml) had marked effects on the cholinergic postsynaptic current. The venom inhibited the peak miniature endplate current (MEPC) amplitude of frog sartorius muscle with slight nonlinearity in the current-voltage relationship. Moreover, it reduced the time constant of MEPC decay (τ_{MEPC}).

The effects of *Philanthus* venom on the glutamatergic synapse were studied using metathoracic flexor and extensor muscles of locust. In the presence of philanthotoxin (0.5 unit), significant voltage dependent depression of the endplate current (EPC) peak amplitude was recorded. There was a marked depression of the dacay time constant of the EPC. The toxin (0.5 unit/ml) depressed the peak amplitude of MEPC and reduced the MEPC time constant.

The present data suggest that the *Philanthus* venom is an inhibitor of the open channel conformation of both nicotinic acetylcholine and glutamate receptors. This study also shows that the venom exerts its paralyzing effect through a central block of the nervous conduction and peripheral block of the neuromuscular system.

Introduction

Natural products have played a key role in the development of several groups of pesticides. In view of this observation, it is likely that a group of insects which use venom to subdue their prey may provide a source of materials with potential as insecticides. One of those insects is the solitary digger wasp (*Philanthus triangulum* F.) a sphecid wasp that preys on honey bee workers. Its venom, which the wasp usually injects into the ventral side of the metathorax into or in the vicinity of the composite ganglion, causes quick paralysis in bees (Fig. 1). An early study suggested that the venom extract did not affect the central nervous system and another mechanism was involved [1]. An analysis of the nature of neuromuscular block in locust casued by

venom exract concluded that it blocked both excitatory and inhibitory transmission, with a concurrent decrease in amplitude of the postsynaptic potentials [2]. Furthermore, a comparison of the effect of *Philanthus* venom on the longitudinal flight muscles of paralyzed and nonparalyzed honey bees led to the suggestion that it blocked the release of neurotransmitters [3]. Neither the crude venom nor its purified toxin were found to affect significantly excitability of the cockroach giant axon [4].

Fig. 1. The solitary digger wasp (*Philanthus triangulum* F.) injects its venom into the ventral side of honey bee worker's thorax causing quick paralysis.

The venom's active component is delta philanthotoxin (δ-PTX) a peptide polyamine (Fig. 2). The venom has been purified, chemically characterized, and subsequently synthesized along with closely related analogues [5,6].

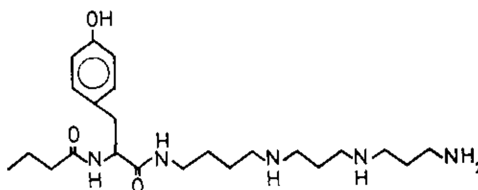

Fig. 2. The chemical structure of philanthotoxin. It has a butyryltyrosyl spermine sequence and a molecular weight of 435.

The major action of δ-PTX was found to be on neurotransmitter receptors, namely, the glutamate, GABA and nicotinic acetylcholine (ACh) receptors. Voltage clamp studies revealed that it was a potent inhibitor of postsynaptic glutamate receptor function in locust leg muscle [7] and was suggested to act on the open receptor channel conformation [8]. It also inhibited cholinergic transmission in the cockroach sixth abdominal ganglion [9] and was very potent on iontophoretically evoked ACh potentials [4]. On rat diaphragm, δ-PTX shortened the decay time of the miniature endplate current [10].

Although there is strong evidence that cholinergic transmission is present in central synapses of arthropods [11-13], investigations of the insect neuromuscular synapse revealed a lack of action of ACh and several other cholinergic agonists and antagonists [14,15]. At the neuromuscular synapses of the arthropods, the neurotransmitter involved in the excitatory process is glutamate [16-18]. The acetylcholine receptor-ionic channel complex (AChR) of the neuromuscular junction, is perhaps the best characterized of the receptors for neurotransmitters. However, little is known about the structure and molecular constituents of the glutamatergic receptor.

Initially this work was directed towards a search for natural compounds, such as philanthotoxin, with activity at neurotransmitter receptors of an insect. Electrophysiological techniques were used in order to determine the mechanism of action of *Philanthus* venom on both nicotinic ACh- and glutamatergic-receptors.

Materials and Methods

Philanthus triangulum females (Fig. 3) were collected from the Dakhla oasis in Egypt. Venom sacs and glands with the sting apparatus attached (Fig. 4) were dissected from ethylacetate-anesthetized wasps, frozen in liquid nitrogen, then lyophilized and stored at-20°C.

Fig. 3. The female of solitary digger wasp *Philanthus triangulum* F. (Order: Hymenoptera, Family: Sphecidae).

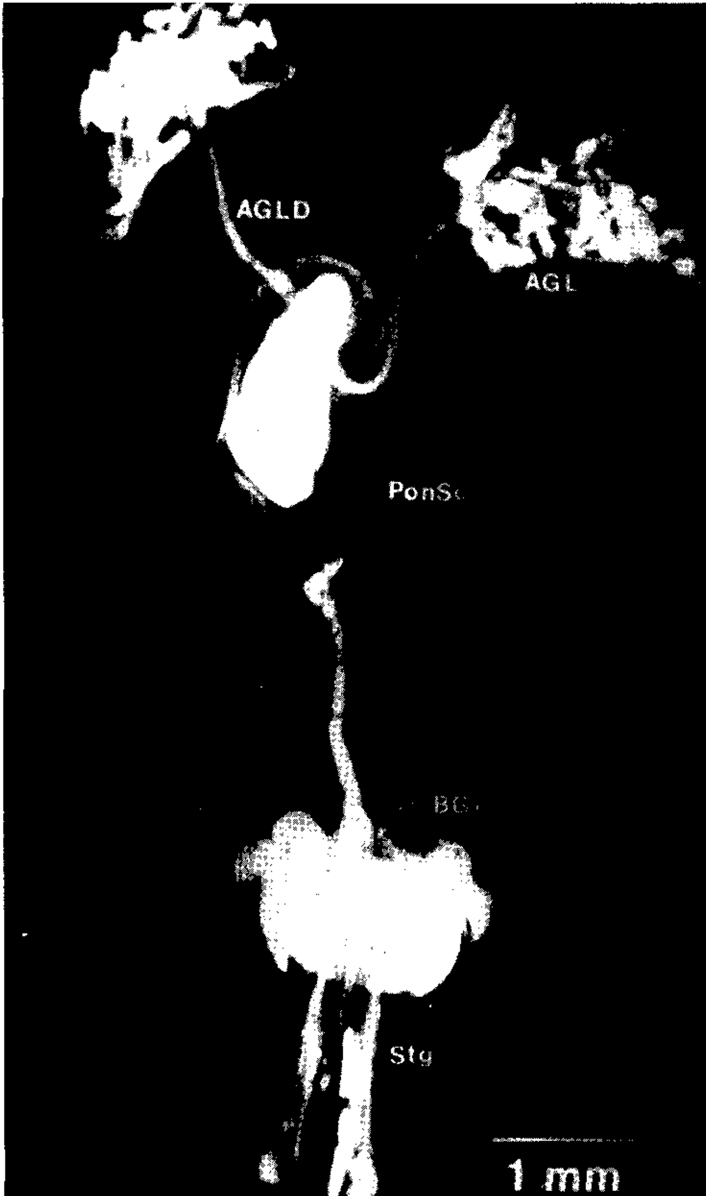

Fig. 4. The sting apparatus and its accessory glands dissected from *Ph. triangulum*. Acidic gland (AGL); acidic gland duct (AGLD); alkaline gland (BGL); venom sac (PonSc) and sting apparatus (Stg).

Philanthus venom extracts

Fifty venom sacs and glands were homogenized in 2 ml of ice-cold glass-distilled water (pH 6) in an all glass hand homogenizer. The homogenate was centrifuged at 30.000 × g for 15 min. The supernatant was saved, and the pellet was rehomogenized in another 2 ml of water and recentrifuged. The two supernatants were pooled, divided in 4 tubes of 1 ml each containing 12.5 units of venom extract (one unit represents the venom extracted from the glands and sac of one wasp) and lyophilized. Injection of one unit into a honey bee produced instant paralysis that lasted for ≅ 30 min. the venom extract was stored lyophilized at -20°C until use, when it was reconstituted in buffers as needed.

Frog muscle preparations

Sartorius muscle preparations of the frog *Rana pipiens* were used for the studies of miniature endplate currents (MEPCs). The frog Ringer's solution had the following composition (mM): NaCl 116, KCl 2, $CaCl_2$ 1.8, Na_2HPO_4 1.3, NaH_2PO_4 0.7, and was saturated with pure oxygen. The final pH of this solution was adjusted to 7.0 ± 0.1. Tetrodotoxin (TTX, 0.3 μM) was added to the bathing medium to prevent twitching during MEPC recording. All the experiments were conducted at room temperature (22-24°C).

Locust nerve-muscle preparations

Flexor and extensor metathoracic tibialis muscles of adult *Locusta migratoria* (Fig. 5) were dissected according to the technique previously described by Hoyle [19] and modified by Idriss *et al.*, [20]. The physiological solution had the following composition (mM): NaCl 170, KCl 10, NaH_2PO_4 4, Na_2HPO_4 6 and $CaCl_2$ 2. [21]. This solution had a pH of 6.8. To decrease the muscle twitch in EPC and EPP experiments, the muscles were treated with glycerol (150 μM), the concentration of $CaCl_2$ was decreased to 0.8 mM, and 10 mM $MgCl_2$ added to the physiological solution [22]. To minimize receptor desensitization, all the preparations were pretreated with 1 μM conanavalin-A for 30 min [23]. All the experiments were carried out at room temperature (22-24°C).

EPC recording and analaysis

The voltage-clamp technique used to evaluate the transient currents generated by the interaction of glutamate with its receptor site was similar to that described by Takeuchi and Takeuchi [24] and modified by Kuba *et al.*, [25]. Glass microelectrodes filled with 3 M KCl and having resistances of 3-5 M Ω were routinely used for intracellular recording and current injection. The EPC waveforms were sent on-line to the computer (PDP 11/40 or 11/24) at a digitizing rate of 10 KHz. The decay phase (80%-20%) was fit by a single exponential (linear regression on the logarithms of the data points) from which the EPC decay time constant (τ_{PEC}) was determined.

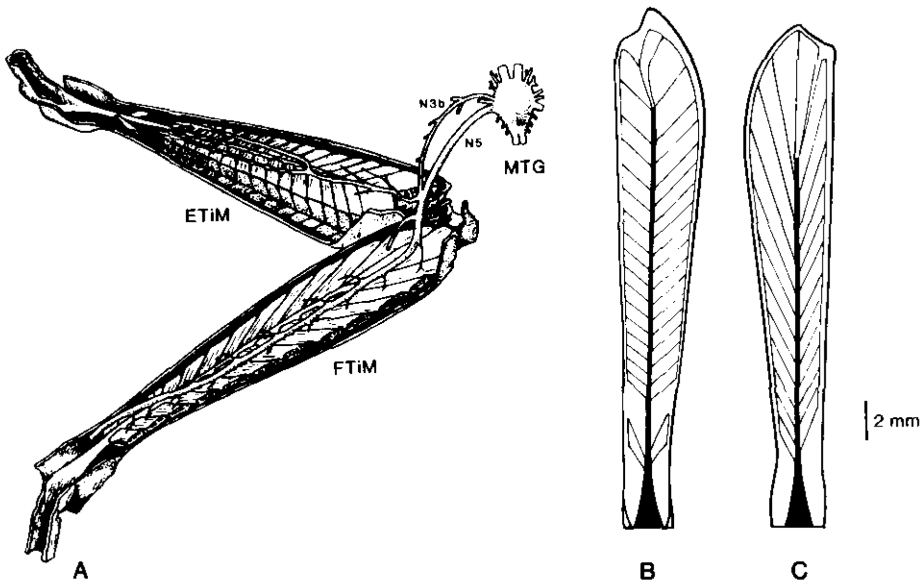

Fig. 5. Three-dimensional view of a dissected locust femur shows the nerve5 (N5) and nerve 3b (N3b) innervating flexor metathoracic tibialis muscle (FTiM) and extensor metathoracic tibialis muscle (ETiM) (A). B and C are schematic drawings of ETiM and FTiM.

MEPC recording and analysis

Miniature endplate currents (MEPCs) were recorded, in the presence of 0.3 μM tetrodotoxin, on magnetic tapes by a Racal Store 4D FM tape recorder for latter analysis on a PDP 11/21 and 11/24 digital computer. MEPCs were filtered at the band width 2.5 KHz using a Krohn-Hite 3700 bandpass filter, captured by a digital oscilloscope (Gould 0S4000) and transmitted to the computer for averaging and analysis.

Statistical analysis

The analysis of the data was performed by using student's t test. p values <0.5 were considered statistically significant.

Results

Effect of philanthotoxin on MEPCs of the frog sartorius muscle

On frog sartorius muscle endplates, the venom produced a concentration and time-dependent depression of the peak amplitude of MEPC and shortening of the MEPC decay time. Fig. 6 shows the effect of the exposure time of philanthotoxin on MEPC peak amplitude and time constant of MEPC decay. The MEPC peak amplitude at a membrane potential of -80 mV was 97.1%, 94,2% and 88.5% control

whereas the τ_{MEPC} was 95.3%, 87.3% and 56.2% of control after 10, 20 and 30 min exposure to the venom (0.04 unit/ml) respectively. At 0.08 unit/ml philanthotoxin, the MEPC peak amplitude and decay time constant were 74.2% and 28.7% of control respectively (Fig. 7). These effects were reversible upon washing the preparation. After 30 min washing, the MEPC peak amplitude and τ_{MEPC} were 100% and 92,7% of control respectively (Fig. 7). MEPC recorded between -60 mV and -100 mV after 30 min exposure to the venom (0.06 unit/ml) showed a very slight non linearity in the peak amplitude- membrane potential relationship (Fig. 8 A). However, the decay time constant of MEPC became clearly less dependent on membrane potential (Fig. 8 B). These results suggested that philantotoxin acted like an open blocker causing noncompetitive blockade of ACh-receptors.

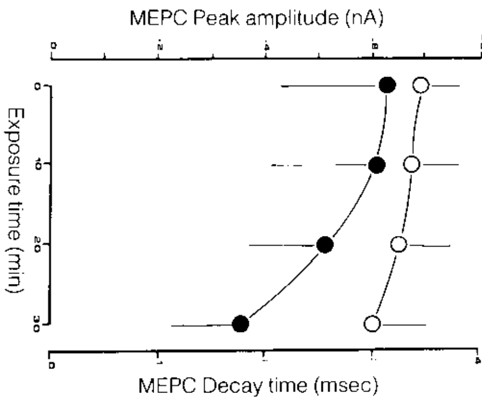

Fig. 6. The effects of philanthotoxin (0.04 unit/ml) on MEPC peak amplitude (opened symbols) and MEPC decay time constant (closed symbols) recorded from frog sartorius muscle at a holding potential-80mV. All the MEPCs were recorded from the same clamped fiber. Each symbol represents the mean ± SD of 17-40 MEPCs.

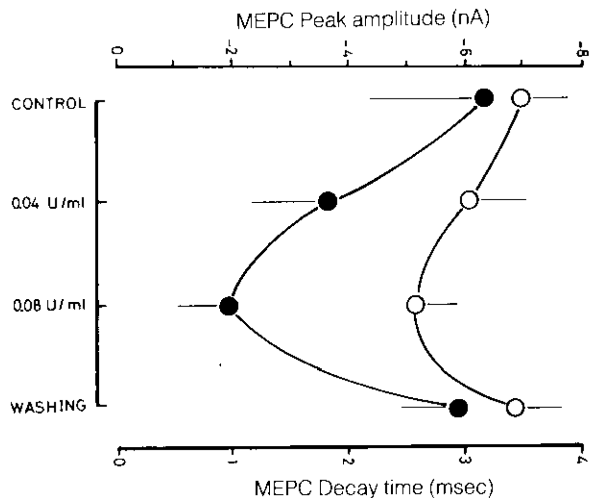

Fig. 7. The effect of 30 min exposure of 0.04 or 0.08 unit philanthotoxin/ml and washing on MEPC peak amplitude (opened symbols) and τ_{MEPC} (closed symbols). Each symbol represents the mean ± SD of 22-37 MEPCs recorded from the same clamped fiber of frog sartorius muscle (holding potential -80 mV).

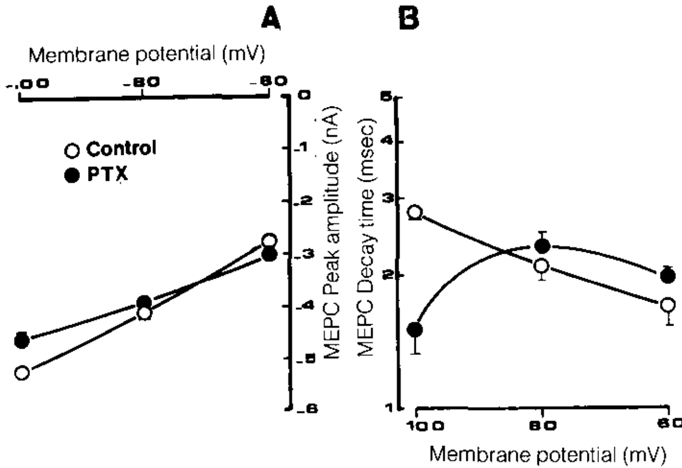

Fig. 8. The effect of philanthotoxin (0.06 unit/ml) on the relationship between MEPC peak amplitude (A) or MePC decay time constant (B) and the membrane potential. Each symbol represents the mean ±SE of 27-42 MEPCs recorded from the same clamped fiber of frog sartorius muscle.

Effects of philanthotoxin of EPc of metathoracic extensor muscle of locust

Studies of glutamatergic synapses have suffered from the relative lack of potent antagonists for the action of glutamate on postsynaptic membranes [9]. The effects of *Philanthus* venom on glutamatergic synapse were studied using voltage clamp technique of the locust leg muscle. The peak amplitude of the control EPC was linear and voltage dependent at potenials between-40 mV and -140 mV. In the presence of philanthotoxin (0.5 unit/ml), significant voltage dependent depression of peak amplitude was recorded but the current voltage relationship remained linear. The effects of venom were reversible upon wash of the muscles (Fig. 9A). Under control conditions, during exposure to *Philanthus* venom and upon washing, the time constant pf EPC decay was voltage independent. There was a marked depression of the decay time constant of the EPC during exposure to the venom (Fig. 9 B).

Effect of philanthotoxin on MEPC of metatharacic flexor muscle of locust

There was a marked depression of peak amplitude of MEPC and the time constant of MEPC decay during exposure to 0.5 unit/ml of *Philanithus* venom. At a holding potential of-60 mV the MEPC peak amplitude was 88.2% and 77.3% of control whereas the τ_{MEPC} was 77% and 64.8% of control after 10 and 20 min exposure to the venom respectively (Fig. 10). The peak amplitude and decay time constant of the MEPC were reversible upon 30 min washing. They were 81.6% and 91.8% of control respectively. From the abovementioned data *Philanthus* venom has also ben shown to inhibit noncompetitively glutamate receptors in locust leg muscle by acting as a channel blocker.

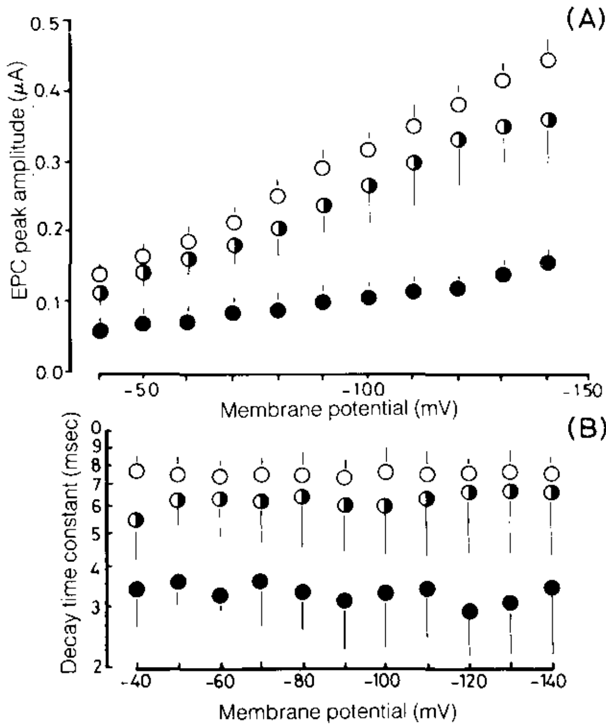

Fig. 9. Effect of *Philanthus* venom on the relationship between EPC peak amplitude of ETiM (A) and its decay time constant (B). Under control condition (○, during exposure to 0.5 unit/ml δ-PTX (●) and after 30 min washing (◑). Each symbol represents mean ± SD of 11-25 EPCs recorded from 4 locust ETiMs.

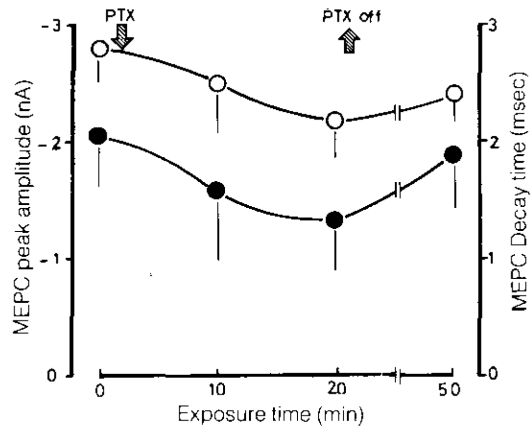

Fig. 10. The relationship between peak amplitude of MEPC (opened symbols) and decay time constant of MEPC (closed symbols) and time of exposure to 0.5 unit/ml δ-PTX. Each symbol represents the mean ± SD of 29-45 MEPCs recorded from clamped fibers of locust FTiM from the same animal. Holding potential was -80 mV.

Discussion

The present study showed that philanthotoxin interacts directly with the nicotinic acetylcholine and glutamate receptor-ionic channel complexes as an open channel blocker.

At neuromuscular junctions, a receptor serves as the recognition site for its neurotransmitter which is released from motor neurons. Binding of neurotransmitter to its receptor induces permeability changes in the postinctional membrane through activition of the ionic conductance modulator (ICM). The ionic conductances that are mediated at neuromuscular junctions by a receptor may be blocked by drugs or toxins that act directly on the receptor (receptor antagonists) or on the ICM [27].

The effects of philanthotoxin on the MEPC recroded of the sartorius muscle in the presence of the venom are proposed to be a result of interaction of the toxin with the ICM in the open conformation (I_o). This may be illustrated in the following hypothetical reaction:

$$I_o + \delta\text{-PTX} \underset{K_{-3}}{\overset{K_3}{\rightleftharpoons}} I_o . \delta\text{-PTX}$$

where K_3 and K_{-3} are rate constants ($K_3 > K_{-3}$). If δ-PTX blocks I_o, the decay time of the MEPC would be determined mainly by the rate of binding the venom to I_o (K_3) and the decay time would be expected to be independent of the membrane potential (Fig. 8B).

From the present experiments it seems that philanthotoxin is capable of blocking synaptic channels opened by glutamate at locust nerve-muscle junction. The venom is able to act on the glutamate receptor ionic-channel complex at concentrations that are high compared with the concentrations affecting the acetylcholine receptor ionic-channel complex.

The present data suggested that the philanthotoxin affects mainly the central nervous system (cholinergic synapse) as an open channel blocker causing paralysis. It also blocks the excitatory neruomuscular junction (glutamatergic synapse) but at relatively high concentrations.

The natural philanthotoxin and its synthetic analogues [5] represent a new additional class of chemicals that are attracting the interest of the pesticide industries for studying its application possibility.

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References

[1] Rathmayer, W. "Das Paralysierungsproblem Beim Bienewolf *Phjilanthus triangulum* F. (Hym. Spec.)." *Z. verlgl. Physiol.*, 45 (1982), 413.

[2] Piek, T., Mantel, P. and Engels, E. "Neuromuscular Block in Insects Caused by the venom of the Differ Wasp *Philanthus triangulum* F." *Comp. gen. Pharmacol.*, 2 (1971), 317.

[3] Piek, T. and Njio, K.D. "Neuromuscular Block in Honeybees by the of the Bee Wolf Wasp (*Philanthus triangulum* F.)." *Toxicon*, 13 (1975), 199.

[4] Piek, T., HUE, B., Pelhate, M., David, J.A., Spanjer, W. and Valdsema Currie, R.D. "Effects of the Venom of *Philanthus triangulum* F. (Hym. Sphecidae) and δ-Philanthotoxin on Axonal Excitability and Synaptic Transmission in the Cockroach CNS." *Arch. Insect. Biochem.*, 1 (1984), 297.

[5] Eldefrawi, A., Eldefrawi, M., Kammo, K., Mansour, N., Nakanishi, K., Oltz, E. and Usherwood, P. "Structure and Synthesis of a Potent Glutamate Receptor Antagonist in Wasp Venom." *Proc. Natl. Acad. Sci.*, 85 (1988), 4910-4913.

[6] Piek, T., Karst, H., Kruk, C., Lind, A., Morle, J., Nakajima, T., Nibbering, N., Shinozaki, H. Sponjer, W. and Tong, Y. "Polyamine Like Toxins a New Class of Pesticides" *Neurotox'88* England, (1988), 7-8.

[7] Spanjer, W., May, T.E., Piek, T. and DE Haan, N. "Partial Purification of Components from the Paralyzing Venom of the Wasp *Philanthus triangulum* F. (Hym. Sphec.) and Their Action on Neuromuscular Transmission in the Locust." *Comp. Biochem. Physiol.*, 710 (1982), 149.

[8] Clark. R.B., Donaldson, P.L., Gration, K.A.F., Lambert J.J., PIEK, T., Ramsey, R., Spanjer, W and Usherwood, P.N.R. "Block of Locust Muscle Glutamate Receptors by δ-Philanthotoxin Ocurs after Receptor Activation." *Brain Res.*, 241 (1982), 105.

[9] Piek, T., Spanjer, W., Veldsema Currie, R.D., Van Groen, T., De Hanna, N. and Mantel, P. "Effect of Venom of the Digger Wasp *Philanthus triangulum* F. on the Sixth Abdominal Ganglion of the Cockroach." *Comp. Biochem. Physiol.*, 71C (1982), 159.

[10] Van Wilgenburg, H., Piek, T. and Mantel, P. "Ion Channel Block in Rat Diaphragm by the Venom of the Digger Wasp *Philanthus triangulum.*" *Comp. Biochem. Physiol.*, 79C. (1984).

[11] Corterggiani, E., and Serfaty, A. "Acetylchline et Cholinesterase Chez les Insects et les Arachnides." *C R Soc. Biol. Biol. (Paris)*, 131 (1939), 1124-1126.

[12] Tobias, J.M., Kollros, J.J., and Savit, J. "Acetylcholine and Related Substances in the Cockroach, Fly and Crayfish, and the Effect of DDT." *J. Cell Copm. Physiol.*, 28 (1946), 159-182.

[13] Colhoun, E.H. "Acetylcholine in *Periplaneta americana* L.I. ACh Levels in Nervous Tissue." *J. Insect Physiol.*, 2 (1958), 117-127.

[14] Colhoun, E.H. "The Physiological Significance of ACh in Insects and Observations Upon other Pharmacologically Active Substances." *Adv. Insect physiol.*, 1 (1963), 1-41.

[15] McDonald, T.J., Farley, R.D., and March, R.B. "Pharmacological Profile of the Excitatory Neuromuscular Synapses of Insect Retractor Unguis Muscle." *Comp. Gen Pharmacol.*, 3 (1972), 327-338.

[16] Usherwood, P.N.R., and Grundfest, H. "Peripheral Inhibition in Skeletal Muscle of Insect." *J. Neurophysiol.*, 28 (1965), 497-518.

[17] Usherwood, P.N.R., and Machili, P. "Pharmacological Properties of Excitatory Neuromuscular Synapese in the Locust." *J. Exp. Biol.*, 49 (1968), 341-361.

[18] Faeder, I.R., and O'Brein, R.D. "Responses of Perfused Isolated Leg Preparations of the Cockroach *Gromphadorhine portentosa* to L-Glutamate, GABA, Picrotoxin, Strychnine and Chlorpromazine." *J. Exp. Zool.*, 173 (1970), 214.

[19] Hoyle, G. "The Anatomy and Innervation of Locust Skeletal Muscle." *Proc. Roy. Soc. London Ser. B.*, 143 (1955), 281-292.

[20] Idriss, M.H., Filbin M.T., Eldefrawi, A.T., Eldefrawi, M.E. and Albuquerque, E.X. "Effects of Chlorisondamine and Philanthotoxin on the Glutamate Receptor/Channel Complex of Locust Muscle." *Fed. Proc.*, 43 (1984), 342.

[21] Idriss, M., and Albuquerque, E.X. "Anticholinesterase (Anti-ChE) Agents Interact with Pre-and Post-Synaptic Regions of the Glutamatergic Synapse." *Biophys. J.*, **47** (1985), 259a.

[22] Idriss, M., and Albuquerque E.X. "Phencyclidine (PCP) Blocks Glutamate-Activated Postsynaptic Currents." *FEBS lett*, 189 (1985), 150-156.

[23] Mathers, D.A., and Usherwood, P.N.R. "Concanavalin a Blocks Desensitization of Glutamate Receptors on Insect Muscle Fibers." *Nature (London)*, 259 (1976), 409-411.

[24] Takeuchi, A., and Takeuchi, N. "Active Phase of Frog's End-Plate Potential," *J. Neurophysiol.*, 22 (1959), 395-411.

[25] Kuba, K., Albuquerque, E.X., Daly, J., and Barnard, E.A. "A Study of the Irreversible Cholinesterase Inhibitor, Disopropylfluorophosphate, on Time Course of End-Plate Currents in Frog Sartorius Muscle." *J. Pharmacol. exp. Ther.*, 189 (1974), 499-512.

[26] Idriss, M. and Albuquerque E.X. "Phencylidine (PCP), a New Potent Antagonist for Glutamate Receptor." *Amer. Soc. Neurosc. Dallas*, TX 844-247 (1985), 11.

[27] Albuquerque, E.X., Eldefrawi, A., Eldefrawi, M., Mansour, N. and Tsai, M. "Amantadine: Neuromuscular Blockade by Suppression of Ionic Conductance of the Acetylcholine Receptor." Science, 199 (1978), 788-790.

سم ذئب النحل: نموذج جديد لمجموعة إضافية من المبيدات الحشرية

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ملخص البحث. سم ذئب النحل مستخلص من أكياس سم ذئب النحل (فيلانثس تراي أنجيولم، ف) هذا السم فعال ضد جميع الحشرات والمادة المؤثرة هي ببتيد عديد الأمين. درس تأثير السم في كل من الشبك الكولينية والجلوتاميتية بواسطة الطرق الكهروفسيولوجية كان لسم ذئب النحل بتركيزات منخفضة (٠,٠٤ وحدة/سم^{٣}) تأثير واضح على التيار البعد شبكي في الجهاز الكوليني. ثبط سم ذئب النحل كل من قمة مدى التيار المصغر المار بالعضلة في منطقة الشبك وكذلك الوقت اللازم لانحلال هذا التيار. كذلك أظهر السم تأثيرا على العلاقة بين التيار والجهد حيث ألغى العلاقة الخطية بين فرق جهد الغشاء وزمن انحلال التيار المصغر المار بالعضلة في منطقة الشبك والمسجل من العضلة الخياطية في الضفدعة.

درس تأثير هذا السم على العضلتين القابضة والباسطة لساق الرجل الخلفية في الجراد كمثال للجهاز الجلوتاميتي. كان هناك تأثير تثبيطي معنوي لسم ذئب النحل بتركيز ٠,٥ وحدة/سم^{٣} على قمة مدى التيار المار بالعضلة في منطقة الشبك وكذلك تأثير مثبط ملحوظ على زمن انحلال هذا التيار. لوحظ التأثير نفسه عند دراسة السم على زمن انحلال التيار المصغر المار بالعضلة في منطقة الشبك.

تشير النتائج الحالية أن لسم ذئب النحل تأثير تثبيطي للقناة المصاحبة لكل من المستقبل الكوليني والجلوتاميتي في وضعها المفتوح. كذلك توضح أن السم يؤدي إلى شلل الحشرة عن طريق تأثيره المباشر في الجهاز العصبي الكوليني والجهاز العصب عضلي الطرفي.