International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2019): 7.583

Effect of Philanthotoxin on Neuromuscular Transmission of Tick *(Rhipicephalus appendiculatus)*

Dr. Tanwir R Khan[1], Dr. Ian R. Duce[2]

[1]Ph.D. (Nottingham), Senior lecturer, Zoology Department, St. Columba's College, Vinoba Bhave University, Hazaribagh 825301, Jharkhand, India

[2]Ph.D. (U.K), Associate Professor, School of Biological Sciences, Nottingham University, Nottingham NG7 2RD, United Kingdom

Abstract: ***Neuromuscular transmission is a main target site for the chemical control of many pests. L-glutamate mediates synaptic excitation at neuromuscular junction in arthropods and regulates skeletal muscle activity. Polyamine amides regulate cell excitability in the neuromuscular system of arthropods. Philanthotoxin (PhTX) and its analogues block the glutamate receptors which are involved in arthropod neuromuscular transmission. In this study the action of synthetic analogues (PhTX-343, C_7-PhTX-343, DNP12-PhTX-343) of PhTX-433 which is an open ion channel blocker were examined on evoked excitatory postsynaptic potential (EPSP) in tick coxal muscle. These compounds all antagonized the evoked EPSP in a use-dependent manner. The tick nerve-muscle preparation has proved to be useful for providing an insight into the numerous and complex interactions that occur between philanthotoxin and glutamate neurotransmission. Philanthotoxin interacts with the open ion channel of GluRs and is potentially useful for the isolation of excitatory amino acid receptors from excitable tissue and for the development of novel pesticides.***

PhTX		Philanthotoxin
NMDA	-	N-Methyl-D-Aspartate
AMPA	-	α-Amino-3-hydroxy-5-methyl-isoxazole propionic acid
GluR	-	Glutamate receptor
qGluR	-	L-quisqualate sensitive glutamate receptor
EPSP		Excitatory Post Synaptic Potential
EAARs	-	Excitatory Amino Acid Receptor-PhTX-343

DNP12- Di-nitro-Phenyl 12-PhTX-343

1. Introduction

Ticks are parasites of vertebrates and although most abundant on mammals and reptiles, they are also common on birds and amphibia. Their food consists entirely of blood and lymph. During the intervening years a large number of ticks have been shown to be vectors of many serious diseases of human and other animals. Ticks constitute the most important livestock pest in Africa, being found on livestock throughout the entire 30 million km^2 of the continent. Ticks are undeniably the most difficult pest and vector to control. Ticks are a major constraint on live stock production with roughly 1.1 million cattle dying annually from theilleriosis alone. In addition tick bites predispose livestock to other diseases and cause enormous production losses through loss of milk, reduced growth rate, reduced calving intervals etc. The acaricides presently available in market are toxic chemicals that have proved unfeasible for tick control. It is urgent to continue the search for new pesticides. It would be preferable to find compound which works at a different site of action from existing commercial pesticides to reduce the likelihood of resistance. Pest biocontrol is becoming one of the most hopeful replacements to chemical pesticides.

This technique is used:

1) To minimize the chemical residues on our planet
2) To minimize the growing problem of arthropod resistance to pesticides
3) To balance rising prices of new chemical pesticides
4) To create friendly environment(chemical free)
5) Due to longer effect of this technique as compared to other methods
6) To overcome the drawback of broad spectrum insecticide

There is considerable evidence for the involvement of glutamate and probably other excitatory amino acids in synaptic transmission. A number of workers have shown that L-glutamate plays a significant role in arthropod neuromuscular transmission (Usherwood and Cull-Candy, 1975: Usherwood 1981; Piek, 1985). Hart (1982) reported that both aspartate and L-glutamate had similar effects on tick leg muscle causing depolarization and abolishing neurally evoked excitatory postsynaptic potential (EPSP). The regulation of arthropod skeletal muscle activity by glutamate gives the receptors for this amino acid a special significance as a target for the chemical control of pests.

Polyamine toxins are potentially useful pharmacological probes possessing a multitude of interesting properties that affect glutamatergic synaptic transmission. The ability of polyamine amides to regulate cell excitability in vertebrate nervous system and the neuromuscular system of arthropods has potential for pharmaceutical and agrochemical applications. Piek *et al.*, (1971) showed that venom of the wasp *Philanthus triangulum* contains a number of toxins including the polyamine toxins, philanthotoxin (PhTX) which block glutamate receptors on locust muscle. Over 100

Licensed Under Creative Commons Attribution CC BY

analogues of PhTX have been synthesized with changes made in the four regions of the structure (Nakanishi *et at.*, 1990). The effect of these analogues of PhTX on insect qGluR were investigated using the locust extensor tibiae muscle (Karst and Piek, 1991) and house fly larval muscle preparation (Benson *et al.*, 1992). Washburn *et al.*, (1996) showed the block of AMPA receptors by PhTX-433. Recently Bowie *et al.*, (1998) reported activity-dependent modulation of glutamate receptor by polyamines. In the present study the effect of PhTX-343, C_7 PhTX-343, DNP12-PhTX-343 (Fig. 1) on tick glutamatergic synaptic transmission were investigated and the potencies of these analogues were determined by studying the changes, that they induced in the excitatory post synaptic potential.

2. Materials and Methods

Adult male ticks *(Rhipicephalus appendicuatus)* were supplied by Schering Agrochemical Plc. Chesterford Park, U.K. The analogues of philanthotoxin (PhTX) were synthesized by Professor Koji Nakanishi and his colleagues at Columbia University, New York. Toxins were diluted in distilled water and the stock solutions were kept at - 20 ^{0}C prior to use.

Un-engorged ticks were pinned ventral side down in a sylgard petri dish containing tick saline (NaCl 200mM; KCl 5mM; $MgCl_2$ 5mM HEPES 2mM; pH 6.9) and a cut was made along the posterior and lateral margins of the sclerotized scutal plate. The scutum was folded anteriorly and the gut, heart and salivary glands were removed with fine forceps to expose the synganglion and coxal muscle (Fig.2a & 2b)The preparation was transferred into a perfusion chamber (0.5 ml) and perfused continuously (3ml/min) with tick saline. Intracellular electrodes (2-5 M□) were inserted through the membrane of single coxal muscle fiber and the cells were allowed to equilibrate for 10 min. The motor nerve supplying the muscle under study was taken up in a suction electrode, which was connected to an isolated stimulator connected to a pulse generator (Fig.3). Pulses were normally delivered at 1 Hz (2-3 Volts, 0.6 ms) through the motor nerve and the evoked excitatory postsynaptic potentials were recorded by using a VCR (Sony, SL-F 25UB PAL) and digital pulse control modulator (Sony, PCM, 701 ES).

3. Results

The resting potential of freshly dissected coxal muscle of *R. appendiculatus* varied betweenyou -50mV to -70mV and the amplitude of neurally evoked EPSPs obtained from coxal muscle varied between -20 mV to -25mV. Miniature excitatory postsynaptic potentials (min-EPSPs) were frequently encountered in the muscles and the low concentration (10^{-8}M) of bath appliedL-glutamate always increased the frequency of min-EPSPs (Fig. 4) while the high concentration (10^{-5} M) eliminated neurally evoked EPSPs in dose- dependent manner (Fig.5). When Octopamine and Acetylcholine was applied at same concentrations no effect was observed on either spontaneous or evoked potentials. Control EPSPs. (n=20) were obtained from coxal muscle and then the muscle preparation was incubated for 10min with different concentrations of PhTX-343 (10^{-4} M to 10^{-6} M) in the absence of motor nerve stimulation and then the toxin was washed out for 30 min with toxin-free tick saline. The evoked EPSPs was not affected. When PhTX-343 was applied during motor nerve stimulation, the amplitude of EPSPs were significantly reduced (Table 1). This observation supports the description of PhTX-343 as an open channel blocker of locust muscle qGluR (May and Piek, 1979; Piek and Spanjer, 1986; Piek *et al.*, 1988).

Dose inhibition data were obtained for PhTX-343, C_7-PhTX-343 and DNP12-PhTX-343 (Dinitro-phenyl 12 PhTX-343) (Fig.6). C_7-PhTX-343 and DNP12-PhTX-343 were more potent (IC_{50} = $5x10^{-8}$M) than PhTX-343 (IC_{50} = $2x10^{-5}$ M). The maximum effect was always observed after 12 min of application (Fig.7). The PhTX antagonized EPSP in use-dependent manner and the recovery of the EPSP following the removal of toxin depend of the concentration of toxin that was applied (Fig.8). When the Ca^{++} concentration in the perfusing bath was raised to 7mM compared to normal physiological Ca^{++} concentration (5mM) the EPSPs always were raised and then declined. This decline in reversal amplitude was not seen when the calcium concentration was raised following 30 min washout of toxin with standard tick saline (Fig.9). To study the channel kinetics, the coxal motor nerve was stimulated between 1 Hz to 8 Hz and the effect of different concentrations of PhTX-343, C_7-PhTX-343, DNP12-PhTX-343 on EPSPs were observed to study the binding of analogues of PhTX with GluR. The data obtained in the presence of different concentration of PhTX clearly suggest that inhibition of EPSP increased when the stimulation was raised (Fig.10). Reduction in EPSP amplitude were not seen when the muscle was stimulated at high frequencies in the absence of toxin. Reversibility from this type of antagonism was difficult to achieve even after 30 min of wash-out with saline.

4. Discussion

The innervation of tick skeletal muscle is very complicated. The general anatomy and histology of the central nervous system of ticks have been studied by Robinson and Davidson (1913). Chow *et al., (*1973) classified the neurons in the brain into two groups, the ganglionic neurons and the motor neurons. Hart *et al.*, (1980) in an ultra-structural study on *Amblyomma variegatum* showed that the synaptic muscle receives a polyneural innervation. Although ultra-structural and electrophysiological studies of insect and crustacean muscles are common, there have been very few such investigations of acarine muscle. The fine structure of skeletal muscle in *Boophilus decoloratus* (Beadle, 1973) and the structure of the neuromuscular junctions of leg muscle and retractor muscle of *Boophilus microplus* and *Amblyomma variegatum* were studied by Hart et al., (1980) and Booth et al., (1985). These authors reported the retractor muscle of *B. microplus* and *A. Variegatum* resembles that of other skeletal muscles previously studied in ixodid ticks. The same author found that the neuromuscular junctions of ticks are similar to those found in other arthropod skeletal muscles. The innervation of neurons were multisynaptic as in the leg muscle of locust (Usherwood, 1972, 1974). They also found that the synaptic vesicles are similar to those of insect and crustaceans (Atwood and Johnston 1968, Osborne

Licensed Under Creative Commons Attribution CC BY

1975). Stimulation of excitatory input to arthropod muscle fiber releases neurotransmitter from motor nerve terminals into the synaptic cleft. The released neurotransmitter binds to the postsynaptic GluR and produces an increase in the ionic permeability of the postsynaptic membrane to K^+, Na^+ and Ca^{++} ions (Anwyl, 1977; Anwyl and Usherwood, 1974). This ionic permeability causes changes in the membrane potential of the postsynaptic muscle and is referred to as an excitatory postsynaptic potential (EPSP). At the neuromuscular junction, the spontaneous release of a quantum of neurotransmitter from motor nerve terminal is known as a miniature excitatory postsynaptic potential (Min-EPSPs). Min-EPSPs were first recorded intracellularly from insect muscle by Usherwood (1961, 1963). In the present study the frequency of min-EPSPs from tick coxal muscle increased when challenged with low concentration (10^{-8} M) of L-glutamate. Usherwood and Machilli (1966), Dowson and Usherwood (1972) and Kerkut and Walker (1966) previously reported that a low concentration of L-glutamate increased the frequency of miniature EPSPs of locust and cockroach muscle fibers. When L-glutamate was applied at high concentration min-EPSPs of coxal muscle of *R. appendiculatus* were inhibited supporting the previous finding of Hart (1982) and Booth et al. (1985). Hart (1982) found that both aspartate and L-glutamate had similar effects on tick leg muscle causing depolarization and abolishing neurally evoked EPSPs. Booth et al., (1985) reported that the retractor muscle of Gene's organ in *Amblyomma variegatum* are probably innervated by glutamatergic neurons. In this study on tick coxal muscle no evidence of fast and slow responses was found, since all potentials recorded had approximately the same time course which supports the finding of Booth *et al.* (1985).

Natural α-philanthotoxin or PhTX-433 and many of its synthetic analogues are efficient, noncompetitive, reversible inhibition of locust muscle qGluR (Eldefrawi *et al.*, 1988). In this study when the tick nerve muscle preparations were incubated in PhTX-343 (10^{-5}M) for 10 min in the absence of motor nerve stimulation and the toxin was then removed, the amplitude of EPSPs (n=20) recorded was not affected by the prior presence of PhTX-343. However the application of PhTX-343 during motor nerve stimulation significantly reduced the EPSP supporting the hypothesis that the philanthotoxin is an open channel blocker of GluR in arthropod muscle (Piek *et al.*, 1971 Clark *et al.*, 1982). The effect of analogues of philanthotoxin, PhTX-343, C_7 PhTX 343 and DNP 12-PhTX-343 (Dinitro phenyl-PhTX-343) was studied on tick (*R. appendiculatus*) neuromuscular junction. Bruce *et. al.*, (1990) and Nakanishi *et. al.* (1990) reported successive increase in the potency of PhTX after increasing the length of chain of the beuty1 group from 4 to 7 and then to 10. The potency of dinitro-phenyl analogue was similar to PhTX-343 when tested on neurally evoked twitch contraction of the locust metathoracic extensor tibiae muscle (Bruce et al., 1990). In this study the tick coxal muscle C_7PhTX-343 and DNP 12 PhTX-343 both exhibited almost the same potency (IC_{50} = 10^{-8}M) and both were more potent than PhTX-343 (IC_{50} = $2x10^{-5}$M). This result suggest that GluR present on tick coxal muscle is more sensitive to DNP12-PhTX-343 and C_7PhTX-343 than those present on locust leg muscle.

The antagonism by philanthotoxin analogues was either reversible or semi reversible and was dependent on the exposure time to toxin and was dependent on the concentration of toxin. The amplitude of the partially antagonized EPSPs exhibited full recovery following a wash of the muscle with toxin free saline whereas the reversibility was incomplete or partial from fully antagonized EPSPs. This study supports the previous finding that antagonism of EPSPs by PhTX was use-dependent (Usherwood 1991). Antagonism of EPSPs by PhTX-343 was transiently reversed (The amplitude of EPSPs initially increased and then started to decline) when Ca^{++} concentration was raised. According to Katz (1969) the exocytotic release of quantal neurotransmitter into synaptic cleft follows a transient influx of Ca^{++} ion through the channels located in the presynaptic membrane of the nerve terminal. Transmitter release from insect *(Calliphora vicina)* glutamatergic motor nerve terminal is Ca^{++} dependent and modulated by presynaptic NMDAR (Antonov and Magazanik, 1999). When the Ca^{++} concentration of tick saline was raised to 7 mM Ca^{++} ions should have flowed down the concentration gradient into the nerve terminal. The increase in intracellular Ca^{++} will result in enhanced release of L-glutamate. The extra released glutamate may open additional qGluR channels resulting in increased EPSP amplitude. However once the GluR channels are open the PhTX-343 will gain access and block them and the resultant block inhibits the EPSPs. The degree of EPSP antagonism was dependent on the exposure time of the preparation to the toxin and the recovery from the antagonism was Ca^{++} dependent. When the Ca^{++} concentration was raised to 7mM after 30 min. washout of toxin with tick saline the EPSPs reversed more quickly. This study suggest that ($[Ca^{++}]i$) may displace toxin from binding site postsynaptically or enhance the presynaptic release of L-glutamate into the synaptic cleft. This release of L-glutamate activates and opens additional qGluR which have not previously been exposed to this neurotransmitter and so increases the EPSP amplitude. When the motor nerve was stimulated at higher frequencies in the presence of PhTX-343 the degree of antagonism was greater. No reduction in EPSP amplitude was seen when the muscle was stimulated at higher frequencies in the absence of PhTX-343. Reversibility from this type of antagonism was difficult to achieve (>15%). On the basis of present data it is confirmed that PhTX is an open ion channel blocker of tick muscle GluR and the binding of PhTX to GluR present on tick muscle depends on channel kinetics. The ability of analogues of PhTX to regulate the neuromuscular transmission of tick has potential for the isolation of EAARs from excitable tissues and for the development of novel acaricides.

References

[1] Antonov, S. M and Magazanik, L. G (1993) Modulation of transmitter release from insect motor nerve ending by NMDA receptors.
[2] Atwood, H.L. and Johnston, H.S. (1968), Neuromusculasynapses of a crab motor axon. J. Exp. Zool. **169**: 457-470.
[3] Anwyl, R. (1977), Permeability of the postsynaptic membrane of an excitatory glutamate synapse to

Licensed Under Creative Commons Attribution CC BY

sodium and potassium. J. Physiol. (London). **273**: 367-388.

[4] Anwyl, R. and Usherwood P.N.R. (1974), Voltage clamps studies of the glutamate synapse. Nature (London) **252**: 591-593.

[5] Beadle, D. J. (1973). Muscle attachment in the tick *Boophilus dectoratus*. Koch (Acarina : Ixodidae). Int. J. insect morphol. Embryol. **2**: 247-255.

[6] Benson, J. A; Schurmann, F : Kaufmann, L; Gsell. L and Piek, T. (1992). Inhibition of dipteran larval neuromuscular synaptic transmission was analogues of Philanthotoxin - 433 : A structure activity. Comp Biochem. Physiol. **102**: 267-272.

[7] Booth, T. F; Beadle, D. J. and Hart, R. J. (1985). An ultra structural and physiological investigation of the retractor muscles of gene's organ in the cattle tick *Boophilus microplus* and *Amblyomma variegatum*. Exp. And Appl. Acarology. **1**: 165-177.

[8] Bowie, D., Lange, G. D., Mayer, M.L. (1998), Activity - dependent modulation of glutamate receptor by polyamines. *J Neuro. Sci***18(20)** : 8175-8185.

[9] Bruce, M; Bukownik, R; Eldefrawi, A. T; Eldefrawi, M. E; Goodnow, R. Jr; Kallimopoulos, T; Nakanishi, K; Miwa, M and Usherwood, P.N.R. (1990), Structure activity relationship of analogues of the wasp toxin philanthotoxin noncompetitive antagonists of quisqualate receptor. Toxicon. **28(11)** : 1333-1346.

[10] Chow, Y. S; Chang, P. C. and Lin, S. H. (1973), Motor neurons map of tropical cattle tick *Zoophiles micro plus* (Acarina : Ixodidae). Int. J. Insect Morph. Embryol. **2** : 305-311.

[11] Clark, R. B; Donaldson, P.L; Gration, K. A.F; Lambert, J. J; Piek, T; Ramsey, R; Spanjer, W and Usherwood, P.N.R. (1982). Block of locust muscle glutamate receptors by - philanthotoxin occurs after receptor activation. Brain Res **241**: 105-114.

[12] Dowson, R. J. and Usherwood, P.N.R. (1972), The effect of low concentration of L-glutamate and L-aspartate on Transmitter release at the locust excitatory nerve-muscle synapse.J.physiol. (London). **299**:13-14.

[13] Eldefrawi, A. T. and Eldefrawi, M. E. (1988). Acetylcholine. In. "Comparative invertebrate neurochemistry". (G. G. Lant and R.W. Olsen eds.) Croom Helm Ltd. PP. 1-41.

[14] Hart, R.J. (1982), Some comparative aspects of the ultrastructure and electrophysiology of insect and tick nervous system (Ph.D. Thesis). Council for National Acad. Award.

[15] Hart, R: J; Beadle, D. J. and Botham, R. P. (1980). The ultrastructure of somatic neuromuscular junction in the tick *Amblyomma variegatum*. Cell. Tissue. Res. **206**: 505-508.

[16] Karst, H; and Piek, T (1991), Structure activity relationship of philanthotoxin-II. Effects on glutamate gated ion channels of the locust muscle fiber membrane. *Comp. Brioche. Physio*. **98C (213):** 479-489.

[17] Katz, B. (1969), The release of neural transmitter substances, Springfield, III. Thomas.

[18] Kerkut, G.A. and Walker, R. J., (1966), The effect of L-glutamate, acetylcholine and GABA on miniature and plate potentials and contractures of the coxal muscles of the cockroach *(Periplanata amricana)Comp. Biochem. Physiol*. **17 :** 435-454.

[19] May, T.E. and Piek, T. (1979), Neuromuscular block in locust skeletal muscle caused by venom preparation made from the digger wasp, *Philanthus triangulamJ. Insect. Physio*. **25** : 685-691.

[20] Nakanishi, K; Goodnow, R; Kalivretenos, A: Choi, S; Fushiya, S; Chiles, G; Eldefrawi, A.T; Eldefrawi, M. E. and Usherwood. P. (1992), Synthetic and Bio-organic study with PhTX analogues In : New leads and target in Drug research. Alfred Benzon symposium 33. Krogsgaard-Larsen, P; Christenses, S.B; Kofod, H.(eds). Mukgaard. Copenhagen PP 138-148

[21] Nakanishi, K; Goodnow. R; Konna, K; Niwa, M; Bukownik, R; Kallimopoulos, T.A; Usherwood. P.N.R; Eldefrawi. A.T. and Eldefrawi, M. E. (1990), PhTX-433 a non competitive glutamate receptors inhibitor. *Pure Appl. Chem***62** : 1223-1230.

[22] Osborne. M. P. (1975). The ultrastructure of nerve muscle synapses. In: Usherwood P(ed). *Insect muscle*. Acad. Press. PP 151-205.

[23] Piek, T; Mantal, P and Engels, E. (1971), Neuro muscular block in insect caused by the venom of the digger wasp *Philanthus triangulum*. F. *Comp. Gen. Pharmacol***2** : 317.

[24] Piek, T. (1985), Neurotransmission and neuromodulation In skeletal muscle in Comprehensive Insect Physiol; Biochem, and Pharm". (Vol. II); Kerkut, G.A. and Gilbert. L.I. (eds) PP : 55-118. Pargamon Press, Oxford.

[25] Piek, T and Spanjer. W. (1986), Chemistry and pharmacology of solitary wasp venoms. In: Piek, T. (ed.) venoms of *Hymenoptera*. Acad. Press. London PP : 161-307.

[26] Piek, T; Fokkens, R. H; Karst. H : Kruk, C; Lind. A : Van Marle, J; Nakajima, T; Nibbering, N. M; Shinozaki, H; Spanjer, W and Tong, Y. C. (1988), Polyamine like toxins-A new class of pesticides ? In Neurotox88 Lunt, G. G(ed.) Elsevier Science Publisher Amsterdam PP 61-77.

[27] Robinson, L. E and Davidson. J. (1914), The anatomy of *Argars persicus* (Oken, 1988), Part 3 : Parasitology. 6 : 382-424.

[28] Usherwood, P.N.R. and Cull-Candy (1975). Pharmacology of somatic nerve-muscle synapses. In "Insect muscle" Usherwood P.N.R. (ed.) pp. 207-280. Academic Press, New York.

[29] Usherwood, P.N.R. (1981). Amino acid gated ion-channels in locust muscle membrane. In Rozsa, K.s (de). Neurotransmission in invertebrates. Adv.Physiol.Sci **Vol.22**:pp 191-203.

[30] Usherwood, P.N.R. (1972). Glutamate as an excitatory transmitter in insect. Neuroscience Res. Prog. Bull. **10 (20)** : 136-143.

[31] Usherwood, P.N.R. (1974). Nerve-muscle transmission at the insect excitatory neuromuscular synapses. *Nature* (London) **210**: 634-636.

[32] Usherwood, P.N.R. and Machilli, P. (1966), Chemical transmission at the insect excitatory neuromuscular synapses. *Nature* (London) **210**: 634-636.

[33] Washburn, M.S. and Dingle dine, R. (1996). Block of alpha-amino-3-hydroxy-5-methyl-4-Isoxazolepropionic acid (AMPA) receptors by polyamines and polyamine toxins. *J. Pharmacol. Exp. Ther*.**278** (2): 669-78.

Licensed Under Creative Commons Attribution CC BY