Presynaptic effects of spider toxins: Increase of high affinity uptake in the arthropod peripheral glutamatergic system

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Summary. In contrast to the reported effects of polyamines on the high affinity neurotransmitter uptake, two polyamine-like spider toxins significantly increase the high affinity uptake of glutamate as demonstrated with high resolution autoradiography. The effects of both spider toxins were compared to those of a polyamine toxin from the wasp *Philanthus triangulum*, which is known to inhibit the high affinity glutamate uptake.
Key words. Spider toxins; glutamatergic transmission; high affinity uptake; high resolution autoradiography.

The search for new insecticides has given rise to an increased interest in the actions of naturally occurring toxins affecting the arthropod peripheral glutamatergic system. These toxins, which are present in the venom of various Hymenoptera and spiders, either paralyze or kill prey. A number of these toxins are low molecular weight substances, all sharing polyamine characteristics. Polyamines have a broad spectrum of physiological activities and a number are known to inhibit selectively the uptake of various amino acid neurotransmitters in the vertebrate CNS [1].

In previous investigations we studied the structure and function of 3 polyamine-like toxins occurring in the venom of the solitary wasp *Philanthus triangulum* F [2-4], β, γ, δ-philantotoxin (β, γ, δ-PTX resp.). One of these toxins (δ-PTX) shows presynaptic as well as postsynaptic activity in the arthropod peripheral nervous system. Postsynaptically it acts as a cation channel blocker, and it could be demonstrated that presynaptically it inhibits the high affinity glutamate uptake in the glutamatergic terminal axons and glia. Moreover, it could be demonstrated that the presynaptic action of δ-PTX is not restricted to arthropod glutamatergic systems; it reduces the high affinity uptake of glutamate in the vertebrate hippocampal formation as well [5].

In this report the presynaptic effects of two spider toxins are described which differ completely from the polyamines investigated previously. The toxins are isolated from the venoms of the Joro spider *Nephila clavata* and the related species *Nephila maculata*. They were chemically characterized [6, 7] as respectively JSTX-3 and NSTX-3; and they have been synthetized as well [8, 9]. For the investigation of their presynaptic effects similar autoradiographic techniques were used as applied to the investigation of the effects of δ-PTX, which techniques allow quantification as well as localization of the accumulated ^{3}H-glutamate.

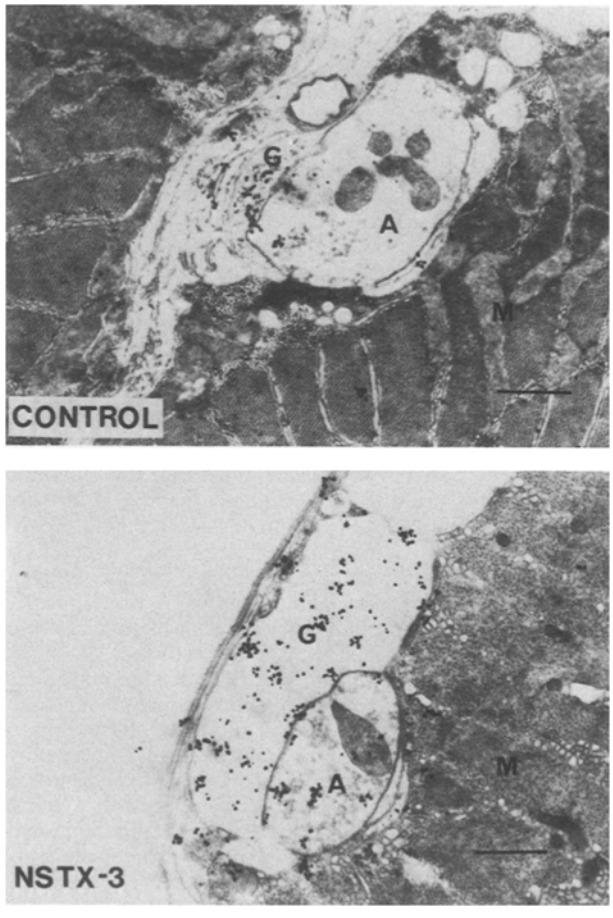
Material and methods. After preincubation of isolated retractor unguis muscles of the locust *Schistocerca gregaria* in an insect saline of 21 °C containing either 100 µM NSTX-3 or 100 µM JSTX-3, during 10 min, the muscles were incubated for 60 min under identical conditions, however the incubation medium contained an additional amount of ^{3}H-glutamate (4.3 µM, 100 µCi/mmol). The insect saline used was composed as follows: NaCl 68, KCl 5, $CaCl_2 \cdot 2H_2O$ 3, $MgCl_2 \cdot 6H_2O$ 5, $NaH_2PO_4 \cdot H_2O$ 6, $NaHCO_3$ 4, glucose 210 (all in mM).

After rinsing in 4 °C saline without glutamate or toxin, the muscles were fixed in glutaraldehyde/OsO_4 and processed for flat substrate high resolution autoradiography. After an exposure period of 10 days the autoradiographs were developed in elon ascorbic acid after gold latensification. For a full description of the technique see van Marle et al. [15, 17]. Each experimental group consisted of 4 muscles. As control 4 muscles were treated similarly, except for the presence of NSTX-3 or JSTX-3 during preincubation and incubation. For each muscle the grain densities above the terminal axons and glia were determined as the average grain density of all terminal axons or glia (at least 12) encountered. Differences between the experimental groups were assessed with the Wilcoxon parameter free test.

Results and discussion. Comparison of the photographs of toxin-treated terminal axons and glia with controls, did not reveal any morphological differences.

No accumulation, no depletion of synaptic vesicles was observed. The muscles did not show any of the degenerative changes sometimes observed after spider bites; albeit that either the period of exposure to the toxins or their concentrations may be too short, respectively too low for these changes to develop.

Analysis of the grain densities above the terminal axons and glia of the NSTX-3 or JSTX-3 treated muscles revealed that



Control and spider toxin (NSTX-3) treated nerve terminals in the retractor unguis muscle of the locust. Note the increased amount of silver grains above the terminal axon and glia in the toxin treated muscle. A, terminal axon; G, glia; M, muscle; scale: 0.5 µm.

Amount of ^{3}H-glutamate, expressed as grain densities (grain/μm^2), accumulated in terminal axons and glia in control and toxin treated retractor unguis muscles

Control		JSTX-3 (100 μM)		NSTX-3 (100 μM)	
t.a.	glia	t.a.$^+$	glia$^+$	t.a.$^+$	glia^{++}
5.6	24.1	14.8	46.6	18.2	52.5
10.6	36.8	19.0	53.3	17.5	32.5
8.0	21.9	14.0	46.1	23.5	55.7
8.0	36.4	13.0	51.2	11.5	41.5
(8.1,2.0)	(29.8,7.9)	(15.2,2.6)	(49.3,3.0)	(15.2,3.0)	(45.5,10.6)*

t.a., terminal axon; $^+$difference from control, $p = 0.014$; $^{++}$difference from control, $p = 0.032$; *($\bar{x}$, SD).

both spider toxins significantly increased the glutamate uptake by terminal axons and glia (fig.). The increase of the amount of ^{3}H-glutamate accumulated was approximately identical for both toxins (table). NSTX-3 and JSTX-3 at a concentration of 100 μM increase the amount of ^{3}H-glutamate in the terminal axons by 100%, the amount of ^{3}H-glutamate accumulated in the glia by ca 55%.

Two biochemically different high affinity glutamate binding systems have been described in the peripheral glutamatergic neuromuscular junctions of arthropods as well as in the vertebrate central nervous system [10–13]. One high affinity binding of glutamate with a K_M of 0.5–10 μM is sodium independent and inhibited by aspartate. This high affinity binding is associated with glutamate-receptor interaction [11–13]. Receptor-bound glutamate cannot be demonstrated with high resolution autoradiography. The other high affinity binding has a somewhat higher K_M (20–50 μM), is sodium-dependent and inhibited by aspartate and chlorpromazine [11, 12]. This high affinity binding system is associated with the removal of glutamate from the synaptic cleft by terminal axon and glia after release from the terminal axon [12–15]; and it is generally assumed that the glutamate accumulated with this high affinity binding system can be demonstrated using high resolution autoradiography.

In previous studies it has been shown that the accumulation of glutamate in the terminal axons and glia is sodium-dependent [15] and affected by both aspartate [15] and chlorpromazine [16]. Moreover, from an analysis of the kinetics of glutamate uptake as demonstrated with high resolution autoradiography a K_M of approximately 15 μM was obtained [17]; which is in close agreement with the biochemical estimates [12, 13]. Therefore we concluded that the glutamate accumulated in terminal axons and glia from an incubation medium containing 4.6 μM glutamate, as demonstrated with high resolution autoradiography, reflects the activity of the sodium-dependent, aspartate- and chlorpromazine-inhibited high affinity glutamate uptake system [15, 17]. Consequently, we consider the increased uptake the result of stimulation of this system by NSTX-3 or JSTX-3 respectively.

Stimulation of the high affinity uptake of glutamate is remarkable since, to the best of our knowledge, no substance has been described as yet, which increases the uptake of amino acid neurotransmitters. The chemically simple polyamines, which were tested in a biochemical assay, reduced the uptake of various amino acids [1]. The beewolf toxin, δ-PTX, reduced the high affinity glutamate uptake significantly [18]; however, from a number of synthetic analogues tested only one appeared to reduce the uptake. The synthetic polyamine toxins MLV 5860 and MLV 6976 did not show any effect on the uptake either [18]. Hence, interference with the uptake system of glutamate seems to be related to a very specific molecular configuration of these polyamines. Consequently, our finding of an increased uptake owing to NSTX-3 and JSTX-3 is surprising.

Electrophysiological experiments revealed that the polyamine δ-PTX has, beside presynaptic effects, important postsynaptic effects.

δ-PTX acts as a cation channel blocker. The combination of presynaptic and postsynaptic effects of the toxin may be the cause of the strongly paralysing activity of this toxin, which we ascribe to a synergism [19]. Electrophysiological experiments for the investigation of postsynaptic effects of NSTX-3 and JSTX-3 in the peripheral as well as the central nervous system are in progress [18]. Preliminary results reveal that the 3 classes of polyamine-like toxins mentioned above, i.e. 1) NSTX-3 and JSTX-3, 2) δ-PTX and its analogues, and 3) MLV 5860 and MLV 6976 respectively induce a reversible postsynaptic block of the glutamatergic arthropod neuromuscular transmission at concentrations between 10^{-8} and 10^{-6} M.

From both the autoradiographic uptake experiments and the electrophysiological experiments we may conclude that the presynaptic and postsynaptic effects of polyamine toxins, as described first for δ-PTX, are based on different entities in the polyamine molecule, since slight differences induce a severe reduction of the uptake inhibition, whereas the postsynaptic block remains unaffected.

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