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Intrahippocampal injection of TsTX-I, a beta-scorpion toxin, causes alterations in electroencephalographic recording and behavior in rats

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ABSTRACT

Aims: TsTX-I scorpion toxin, also known as γ-toxin, is a β-toxin which binds to site 4 of the sodium channel, shifting its activation potential. There are few studies about its pharmacological action in the central nervous system. The objective of this work was to determine the electroencephalographic, behavioral and histopathological effects of intrahippocampal injection of TsTX-I.
Main methods: Rats were anesthetized and fitted with cannulae for injection into the hippocampus and with electrodes for cerebral recording. The animals were treated with Ringer solution, some doses of TsTX-I, DMSO 0.1% or veratridine. Behavioral and electrographic recordings were observed for 4 hours after the injection. After 7 days, the rats were perfused, and their brains removed for histological analysis.
Key findings: Increasing doses of the toxin evoked epileptic-like discharges, wet dog shakes, and in some cases hind limb paralysis and intense respiratory difficulty followed by death. The histopathological analysis demonstrated no cell loss. Animals injected with veratridine developed epileptiform activity in the electrographic recording and neuronal loss.
Significance: The results suggest that TsTX-I toxin may be responsible, at least in part, for the epileptic and behavioral effects observed with the crude venom, and although veratridine and TsTX-I act on Na-channel, the differences between them are remarkable, demonstrating that toxins can have different functional effects depending on the site of action in the channel. Thus, animal neurotoxins are often highly selective and may be useful for the identification of the sequence of events underlying neurotransmission.

Introduction

Scorpion venoms are a mixture of components such as mucopolysaccharides, serotonin and histamine, protease inhibitors (Couraud and Jover 1983), and neurotoxins. These neurotoxins are the best studied components (Possani et al. 1999) and are basic polypeptides that recognize ion channels and receptors of excitable membranes (Catterall 1980; Gordon et al. 1998) inducing an intense autonomic discharge, leading to a massive release of neurotransmitters (Ismail 1995; Possani et al. 1999). All known Na^+-channel-specific toxins from scorpion venom are composed of 60–76 amino acid residues and are stabilized by four disulfide bridges (for review see De la Vega and Possani 2005). These neurotoxins are modifiers of the channel gating mechanism and can be classified into two types: α- and β-scorpion neurotoxins, which bind to external receptor sites 3 and 4 of voltage-gated Na^+ channels, respectively (De la Vega and Possani 2005; Vasconcelos et al. 2005). α-Neurotoxins slow or block sodium channel inactivation. β-Neurotoxins induce both a shift in the voltage dependence of sodium channel activation in the hyperpolarizing direction and a reduction in the peak sodium current amplitude. The differences between α- and β-scorpion neurotoxins with regard to structure and action on Na^+ channels have been extensively investigated, but the differences regarding pharmacological effects are less known (Vasconcelos et al. 2005).

TsTX-I, a β-scorpion toxin, also called Ts1 or toxin-γ (Possani et al. 1977; Sampaio et al. 1991; Becerril et al. 1997) is the major toxin from *Tityus serrulatus* venom (Vasconcelos et al. 2005) and is also the best studied. Several studies have demonstrated its peripheral action such as pre-junctional sensitization of the guinea pig vas deferens (Sampaio et al. 1983; Arantes et al. 1989), increase in acetylcholine output through the activation of Na^+ channels in the motor nerve terminals (Oliveira et al. 1989), increase in the volume, acidity and pepsin output of rat stomach (Cunha-Melo et al. 1991), prolongation of the hypotensive effect in anesthetized rats (Sampaio et al. 1991), reduction in the contractile force of isolated rat atria (Couto et al. 1992) and increase in pancreatic exocrine secretion

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(Possani et al. 1991; Fletcher et al. 1996). More recently, it was demonstrated that this toxin causes a concentration- and time-dependent increase in the release of norepinephrine and epinephrine from bovine adrenal medullary chromaffin cells (Conceição et al. 1998) and produces a selective release of purines from postganglionic sympathetic nerves in the rat vas deferens without modifying the overflow of noradrenaline (Conceição et al. 2005). Nevertheless, the literature lacks studies about the effect of this toxin on the central nervous system. Thus, the present work aimed to assess the effects evoked by TsTX-I, when injected into the hippocampus of rats. For comparison, we used veratridine (VTD), an alkaloid from lilaceous plants that binds to site 2 of the sodium channel (Bicalho et al. 2002) which is believed to cause epileptiform discharges (Link et al. 2008) and depolarization-induced neurotoxicity (Akasofu et al. 2008).

Materials and methods

Subjects

Male Wistar rats (230–250 g) were used. Upon their arrival in the laboratory, the rats were individually housed in wire mesh cages and maintained in a room with constant temperature (22 ± 1 °C), on a 12L:12 D cycle, with food and water provided ad libitum. The animals used in this study were maintained in accordance with the guidelines of the Department of Pathology, São Paulo University's School of Veterinary Medicine, which follows the guidelines for animal care prepared by the Committee on Care and Use of Laboratory Animal Resources, National Research Council, USA. All the experimental procedures were conducted with prior permission from our Institutional Ethics Committee for Experiments on Animals (Protocol No. 215/02).

Chemicals

TsTX-I scorpion toxin (Butantan Institute, São Paulo, SP, Brazil), Ringer solution (Aster, Sorocaba, SP, Brazil) in which the toxin was dissolved, veratridine (VTD, Sigma, St. Louis, MO, USA), and dimethyl sulfoxide 0.1% (DMSO, Sigma, St. Louis, MO, USA) in which VTD was dissolved.

Surgery

The animals were anesthetized (3 ml/kg) with a mixture of pentobarbitone (1.0 g) and chloral hydrate (4.0 g) in 100 ml of 0.9% NaCl and submitted to stereotaxic surgery. Stainless steel guide cannulae were chronically implanted in the dorsal hippocampus for the intracerebral injection, and bipolar twisted electrodes were positioned on the other side of the dorsal hippocampus for depth recordings. Coordinates were derived from a stereotaxic atlas (Paxinos and Watson 1998). Surface recordings were obtained with jeweler's screws positioned bilaterally over the occipital cortex. An additional screw placed in the frontal sinus served as a reference (indifferent electrode). After surgery, animals were housed individually and were allowed to recover for a period of 1–2 days.

Experimental groups and drug administration

The rats were divided into 8 groups with 8 animals in each, and submitted to intrahippocampal injection of 1 μl of Ringer solution, 1 μl of TsTX-I toxin (0.625, 1.25, 2.5, 5.0 or 10.0 ng/μl), 1 μl of 500 μM VTD or 1 μl of 0.1% DMSO, respectively.

Electroencephalographic recordings and behavioral observations

Electroencephalographic recordings and behavioral observations were carried out in a glass compartment. After 15 min of habituation to the test cage, the baseline electroencephalographic recording was taken for 15 min, and the animals were then injected. Next, continuous electroencephalographic recording and observation of animal behavior were performed for a period of 4 h. Fisher's test was used to analyze all the study parameters ($p<0.05$).

Histology

Morphological analysis was performed 7 days after the injection. The animals were deeply anesthetized with CO_2 and perfused through the heart (left ventricle) with phosphate-buffered saline (PBS) followed by 10% formalin solution. The brains were removed, stored in formalin for at least one week, and embedded in Paraplast®. Coronal brain sections of 10 μm were cut from a 700-μm brain block containing the cannula track. The slices were mounted on a glass slide and stained with cresyl violet. The number of cells in the CA1, CA3 and CA4 hippocampal areas was determined by light microscopy using a 40× objective. A two-dimensional cell count was performed using a 100 × 100 μm grid. Only pyramidal neurons located inside the grid area, with a visible nucleus and nucleolus, were considered intact. ANOVA followed by Tukey's test was used for statistical analysis ($p<0.05$).

Results

Electroencephalographic recordings and behavioral observations after intrahippocampal injection

Intracerebral injection of Ringer solution or 0.1% DMSO did not alter the behavior or the electroencephalographic recording (Table 1).

Veratridine evoked wet dog shakes (WDS) and epileptiform discharges of short or long duration in all the animals. These alterations appeared immediately after the injection and lasted only during the first hour of the recording. There were no respiratory alterations or lethality in the animals (Table 1).

Intrahippocampal injection of the toxin induced seizures at all the doses tested, alternating between polyspikes and epileptiform discharges of short or long duration, which began immediately after

Table 1
Electrographic and behavioral effects observed after intrahippocampal injection of Ringer, 0.1% DMSO, VTD or different doses of TsTX-I in rats.

	Epileptic-like discharge	WDS	Myoclonus	Respiratory difficulty	Nasal bleeding	Hind limb paralysis	Death
Ringer (n = 8)	0/8	0/8	0/8	0/8	0/8	0/8	0/8
DMSO, 0.1% (n = 8)	0/8	0/8	0/8	0/8	0/8	0/8	0/8
VTD, 500 μM (n = 8)	8/8*	8/8*	0/8	0/8	0/8	0/8	0/8
TsTX-I 0.625 ng/μl (n = 8)	3/8	0/8	0/8	0/8	0/8	0/8	0/8
TsTX-I 1.25 ng/μl (n = 8)	8/8*	8/8*	0/8	2/8	0/8	0/8	0/8
TsTX-I 2.5 ng/μl (n = 8)	8/8*	8/8*	2/8	3/8	0/8	0/8	0/8
TsTX-I 5.0 ng/μl (n = 8)	8/8*	8/8*	0/8	4/8	1/8	4/8	0/8

Data represent the number of animals that showed the occurrence in relation to the total number of animals in the group. Fisher's test *$p<0.05$, significantly different from control group.