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A microdialysis study of glutamate concentration in the hippocampus of rats after TsTX toxin injection and blockade of toxin effects by glutamate receptor antagonists

Ana Leonor A. Nencioni[a,b], Ivo Lebrun[c], Valquiria A.C. Dorce[a,*]

[a]*Laboratory of Pharmacology, Butantan Institute, Av. Dr. Vital Brasil, 1500, 05503-900 São Paulo, SP, Brazil*
[b]*Laboratory of Pharmacology, School of Veterinary Medicine, São Paulo University, 05508-900 São Paulo, SP, Brazil*
[c]*Laboratory of Biochemistry and Biophysics, Butantan Institute, Av. Dr. Vital Brasil, 1500, 05503-900 São Paulo, SP, Brazil*

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Abstract

Scorpion toxins act on ionic channels changing the release of neurotransmitters. In the present study, we investigated the glutamatergic release evoked by intrahippocampal injection of TsTX toxin isolated from *Tityus serrulatus* scorpion venom in male Wistar rats and the blockade of the toxin effect by glutamatergic antagonists. Microdialysis for neurotransmitter level quantification, electroencephalographic recording, and histopathological analysis were performed. The microdialysis method revealed enhanced levels of extracellular glutamate in the hippocampal area. The toxin injection preceded by injection of the glutamate receptor antagonists dizolcipine maleate (MK-801), D(−)2-amino-5-phosphonopentanoic acid (AP-5), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), L(+)-2-amino-3-phosphonopropionic acid (AP-3), and (+)-α-methyl-4-carboxyphenylglycine (MCPG) demonstrated that MK-801 and AP-5 fully blocked the electrographic alterations and the CA1 cell loss induced by the toxin. CNQX, AP-3, and MCPG partially blocked the epileptiform discharges and no hippocampal damage was observed. Thus, we conclude that the toxin evokes glutamate release and that glutamate receptor antagonists can partially or totally block the toxin effect.

Keywords: Scorpion toxins; Excitatory amino acids; Glutamate receptor antagonists; Hippocampus; Neurodegeneration

1. Introduction

The toxicity of scorpion venom is due to the neurotoxins it contains. These toxins are basic polypeptides with molecular weights of approximately 7000, with no enzymatic action (Couraud and Jover, 1983) and have been shown to affect the ion permeability of excitable cells (Catterall, 1980). They act on distinct ion channels such as sodium (Catterall, 1980; Rochat et al., 1979), potassium (Carbone et al., 1982; Miller et al., 1985), chloride (DeBin et al., 1993), and calcium (Valdivia et al., 1992).

Scorpion neurotoxins that act on sodium channels have been divided into two groups, α and β toxins, according to the ligand-binding sites in the channel. The α-scorpion toxins were the first to be studied and their primary effect is interference with the ability of channels to be inactivated upon prolonged depolarization, with increased sodium permeability and thus increased duration of the action potential (Kirsch et al., 1989). One of their main effects is neurotransmitter release from neuronal endings (Couraud and Jover, 1983).

Several scorpion toxins from *Tityus serrulatus* venom were isolated by many groups, which used different standards to denominate them. In order to uniformize the nomenclature, Sampaio et al. (1991) proposed a nomenclature that assigns a single name to the toxins shown to be identical and this was the nomenclature adopted in the present study.

In the present investigation, we used TsTX toxin (according to Sampaio et al., 1991) previously denominated TS-8F (Carvalho et al., 1998), an α-toxin (Becerril et al., 1997) with 61 amino acid residues (Carvalho et al., 1998) with sequence

* Corresponding author. Tel.: +55-11-3726-7222x2299; fax: +55-11-3726-1505.
E-mail address: vdorce@usp.br (V.A.C. Dorce).

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homology with toxin IV-5 isolated by Possani et al. (1981). The same toxin has been already isolated by other authors and has been variously named T_2III (Sampaio et al., 1983), X_4 (Arantes et al., 1989), TsTx (Sampaio et al., 1991), IV (Mansuelle et al., 1992), and Ts3 (Becerril et al., 1997).

It was demonstrated that this toxin modifies neurotransmitter release (Lima and Freire-Maia, 1977; Gomez et al., 1995) and recent studies have demonstrated that this toxin evokes glutamate release from cortical synaptosomes of rats (Fletcher et al., 1996; Massensini et al., 1998).

The effects obtained after the intrahippocampal injection of TsTX (Carvalho et al., 1998) appear to be similar to those observed after intrahippocampal injection of kainic acid (Cavalheiro et al., 1982) and are characterized by cellular loss and gliosis. This fact suggests an involvement of glutamate receptors in the toxin action. Therefore, we proposed to investigate the in vivo change in glutamate release after toxin injection and the possible blockade of the toxin action using excitatory amino acid antagonists.

2. Materials and methods

2.1. Subjects

Male Wistar rats weighing 200–250 g were used. Upon their arrival to the laboratory (7 days before the experiments), the rats were individually housed in wire mesh cages and maintained in a room with constant temperature (22 ± 1 °C), on a 12L:12D cycle (lights on at 0700 h), 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 at the São Paulo University School of Veterinary Medicine, which follow the guidelines for animal care prepared by the Committee on Care and Use of Laboratory Animal Resources, National Research Council, USA.

2.2. Chemicals

TsTX scorpion toxin (Butantan Institute, São Paulo, SP, Brazil), Ringer's solution (Aster, Sorocaba, SP, Brazil), the competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist D(−)2-amino-5-phosphopentanoic acid (AP-5; Research Biochemicals Int., Natick, MA, USA), the non-competitive NMDA receptor antagonist dizocilpine maleate (MK-801; Research Biochemicals Int.), the ±-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; Research Biochemicals Int.), the metabotropic glutamate receptor antagonists (+)-α-methyl-4-carboxiphenylglycine (MCPG; Research Biochemicals Int.), and L(+)-2-amino-3-phosphonopropionic acid (AP-3; Research Biochemicals Int.) were used. TsTX toxin, MK-801, AP-5, and CNQX were dissolved in Ringer's solution. AP-3 and the MCPG were dissolved in Ringer's solution and 1 N NaOH.

2.3. Surgery

Rats were anesthetized (3 ml/kg) with a mixture of pentobarbitone (1.0 g) and chloralhydrate (4.0 g) in 100 ml of 0.9% NaCl, and positioned in a stereotaxic frame. In animals submitted to microdialysis, guide cannulas were chronically implanted into one side of the dorsal hippocampus and fixed with dental acrylate. The same guide cannula was used for the injection of toxin and for the probe. Coordinates were derived from the Atlas of Paxinos and Watson (1998) (AP −5.3, L −4.0, V −2.0). In the other groups of animals, stainless steel guide cannulas were chronically implanted and fixed with dental acrylate into one side of the dorsal hippocampus for the intracerebral injections and coordinates were AP −4.8, L −3.2, V −2.5. For depth recordings, bipolar twisted electrodes were positioned on the other side of the dorsal hippocampus and were anchored to the skull with dental acrylate. Coordinates were AP −4.8, L 3.2, V −3.0. Surface recordings were obtained with jeweler screws positioned bilaterally over the occipital cortex. An additional screw placed in the frontal sinus served as reference (indifferent electrode). After surgery, animals were housed individually and were allowed to recover for a period of 1–2 days.

2.4. Experimental groups and drug administration

2.4.1. Extracellular amino acid levels

Subjects were injected with TsTX at the dose of 1.0 μg/rat administered intrahippocampally in an injection volume of 1.0 μl/rat ($n = 8$).

2.4.2. Electroencephalographic recordings and behavioral observations

Subjects were divided into 12 groups: (i) injected with Ringer's solution administered intrahippocampally in an injection volume of 1.0 μl/rat ($n = 4$), (ii) injected with TsTX at a dose of 1.0 μg/rat administered intrahippocampally in an injection volume of 1.0 μl/rat ($n = 8$), (iii) injected with MK-801 at a dose of 1.0 mg/kg administered intraperitoneally in an injection volume of 1.0 ml/kg ($n = 4$), (iv) injected with MK-801 at a dose of 1.0 mg/kg administered intraperitoneally in an injection volume of 1.0 ml/kg 30 min prior to the toxin ($n = 5$), (v) injected with AP-5 at a dose of 1.0 μg/rat administered intrahippocampally in an injection volume of 1.0 μl/rat ($n = 4$), (vi) injected with AP-5 at a dose of 1.0 μg/rat administered intrahippocampally in an injection volume of 1.0 μl/rat 45 min prior to the toxin ($n = 6$), (vii) injected with CNQX at a dose of 2.0 μg/rat administered intrahippocampally in an injection volume of 1.0 μl/rat ($n = 6$), (viii) injected with CNQX at a dose of 2.0 μg/rat administered intrahippocampally in an injection volume of 1.0 μl/rat 20 min prior to the toxin ($n = 6$), (ix) injected with AP-3 at a dose of 0.1 mg/rat administered intrahippocampally in an injection volume of 2.0 μl/rat ($n = 5$), (x)

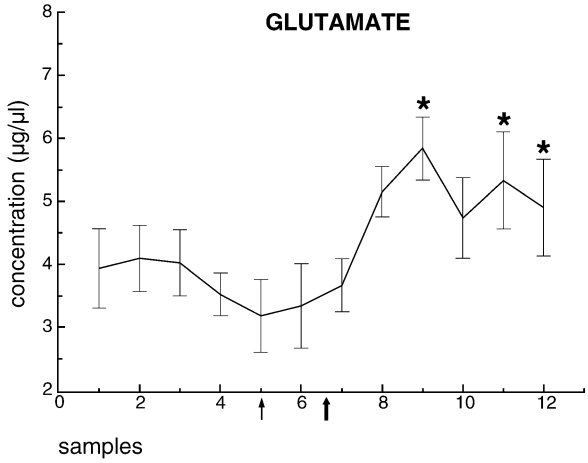

Fig. 1. Extracellular levels of glutamate before (Samples 1–6) and after (Samples 7–12) intrahippocampal injection of TsTX (1.0 μg/μl) in rats ($n=8$). The thin arrow denotes the injection time and the thick arrow denotes the beginning of alterations after toxin injection (because the perfusate required 45 min to go through the outlet polyethylene tubing). Data shown are mean ± S.E.M. Data were analyzed statistically by one-way ANOVA for repeated measures and by Tukey's post hoc test ($P<.05$).

injected with AP-3 at a dose of 0.1 mg/rat administered intrahippocampally in an injection volume of 2.0 μl/rat 30 min prior to the toxin TsTX ($n=7$), (xi) injected with MCPG at a dose of 0.2 mg/rat administered intrahippocampally in an injection volume of 3 μl/rat ($n=8$), (xii) injected with MCPG at a dose of 0.2 mg/rat administered intrahippocampally in an injection volume of 3 μl/rat 30 min prior to the toxin ($n=6$).

2.5. Microdialysis

The experiment was performed on freely moving rats 24 h after guide cannula implantation. Probes (CMA/11 microdialysis probes, Stockholm, Sweden; membrane length 4 mm) were introduced into the guide cannula. A Hamilton syringe (2.5 ml) was connected to the inlet cannula of the probe with a 40-cm length of polyethylene tubing. Ringer's solution was used as perfusion fluid at a flow rate of 1.52 μl/min. The actual flow rate was 1.33 μl/min due to the dead volumes of the perfusion system. The outlet cannula of the probe was connected to a 30-cm length of polyethylene tubing whose internal volume was 2 μl/cm. Thus, approximately 45 min were necessary for the perfusate to go through the catheter and to be collected into a 0.5-ml tube. After a 45–50-min equilibration period, perfusates were collected every 30 min. After four perfusates, which were used to determine the basal extracellular amino acid levels in the hippocampus, the probe was disconnected without flow interruption and toxin was injected through the cannula. The probe was then connected again and eight microdialysis samples were collected until the end of the experiment.

2.6. Amino acid analysis

All samples were analyzed for amino acid content using high-performance liquid chromatography (HPLC) along with fluorometric detection after precolumn derivatization with phenyl-isothiocyanate. The mobile phase consisted of solution A (19 g sodium acetate, 0.5 ml triethylamine, and 100 mM ethylenediaminetetraacetic acid [EDTA]; to 470 ml of this solution, 30 ml acetonitrile was added), and solution B (400 ml of acetonitrile plus 100 ml of water and 100 μl of 1 M EDTA). The gradient profile was determined to clearly identify each amino acid sample by comparing with the retention time of an amino acid standard solution containing 1 mM glutamate, 1 mM γ-aminobutyric acid (GABA), and 1 mM glycine. The sample volume needed to analyze amino acid content was 150 μl obtained after adding the phenyl-isothiocyanate to the 45-μl sample volume.

Extracellular glutamate, glycine, and GABA levels in the dialysate were expressed as μg/μl. Statistical analyses were

Table 1
Percentage of animals that showed electrographic and behavioral alterations after injection of antagonists with or without toxin injection

Treatment	Electrographic alterations			
	Isolated spikes (%)	Short epileptic-like discharges (%)	Long epileptic-like discharges (%)	Clustered slow waves (%)
Ringer's ($n=4$)	0	0	0	0
TsTX ($n=8$)	0	0	100 *	0
MK-801 ($n=4$)	0	0	0	66.6 *
MK-801 + TsTX ($n=5$)	0	0	0	66.6 *
AP-5 ($n=4$)	0	0	0	0
AP-5 + TsTX ($n=6$)	33.3	0	0	0
CNQX ($n=6$)	0	0	0	0
CNQX + TsTX ($n=6$)	33	0	33	0
AP-3 ($n=5$)	0	0	0	0
AP-3 + TsTX ($n=7$)	0	28.5	0	0
MCPG ($n=8$)	0	0	0	0
MCPG + TsTX ($n=6$)	0	16.6	0	0

Treatment	Behavioral alterations			
	Immobility (%)	Postural loss (%)	Orofacial movements (%)	Wet dog shakes (%)
Ringer's ($n=4$)	0	0	0	0
TsTX ($n=8$)	100 *	0	100 *	100 *
MK-801 ($n=4$)	0	33.3	0	0
MK-801 + TsTX ($n=5$)	0	83.3 *	0	0
AP-5 ($n=4$)	0	0	0	0
AP-5 + TsTX ($n=6$)	0	0	0	33.3
CNQX ($n=6$)	0	0	0	0
CNQX + TsTX ($n=6$)	0	0	0	33
AP-3 ($n=5$)	0	0	0	0
AP-3 + TsTX ($n=7$)	0	0	0	28.5
MCPG ($n=8$)	0	0	0	0
MCPG + TsTX ($n=6$)	0	0	0	16.6

* $P<.05$ is considered significant when compared with the control group (Ringer's). Fisher's test.

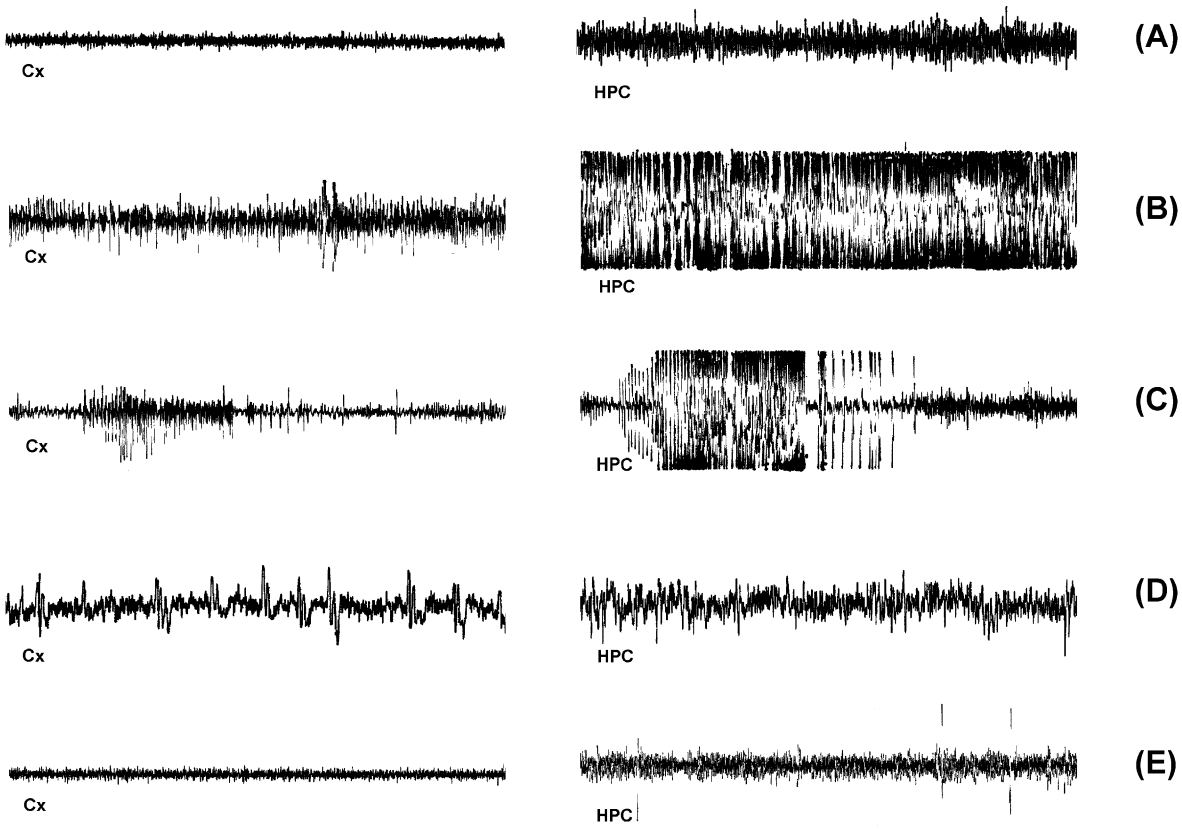

Fig. 2. Examples of epileptiform parameters observed during electrographic activity recording after intrahippocampal injection in control or experimental groups. (A) Normal activity, (B) long epileptic-like discharge, (C) short epileptic-like discharge, (D) clustered slow waves, (E) isolated spikes. Cx, cortex; HPC, hippocampus. Fisher's test was used to analyze all the parameters observed ($P < .05$).

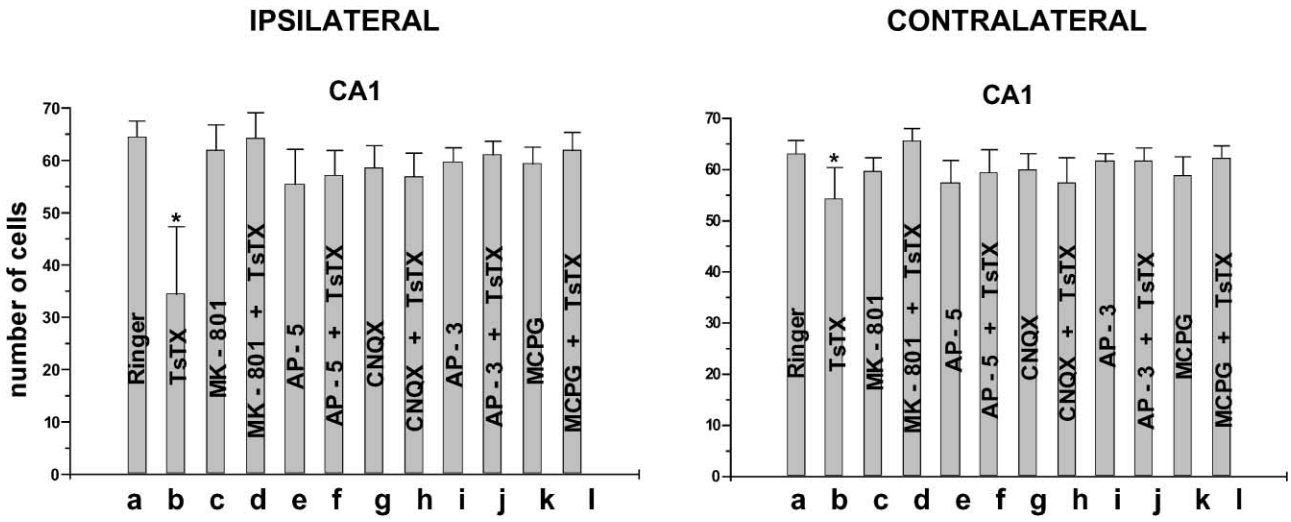

Fig. 3. Brain sections (10 μm) from perfusion-fixed rats were analyzed by light microscopy. Analyses concerned the pyramidal layer of the CA1 hippocampal area of treated (ipsilateral) and contralateral sides. (a) Intrahippocampal Ringer's, 1.0 μl/rat ($n = 4$); (b) intrahippocampal TsTX, 1.0 μg/μl ($n = 8$); (c) intraperitoneal MK-801, 1.0 mg/kg ($n = 4$); (d) intraperitoneal MK-801, 1.0 mg/kg 30 min prior to the toxin ($n = 5$); (e) intrahippocampal AP-5, 1.0 μg/μl ($n = 4$); (f) intrahippocampal AP-5, 1.0 μg/μl 45 min prior to the toxin ($n = 6$); (g) intrahippocampal CNQX, 2.0 μg/μl ($n = 6$); (h) intrahippocampal CNQX, 2.0 μg/μl 20 min prior to the toxin ($n = 6$); (i) intrahippocampal AP-3, 0.1 mg/2.0 μl ($n = 5$); (j) intrahippocampal AP-3, 0.1 mg/2.0 μl 30 min prior to the toxin ($n = 7$); (k) intrahippocampal MCPG, 0.2 mg/3.0 μl ($n = 8$); (l) intrahippocampal MCPG, 0.2 mg/3.0 μl 30 min prior to the toxin ($n = 6$). Data are represented as means ± S.D. number of cells. Statistically significant changes within the control group (Ringer's) and experimental groups were evaluated by ANOVA followed by Tukey's test. * denotes $P < .05$.

carried out by one-way analysis of variance (ANOVA) for repeated measures and Tukey's post hoc test ($P < .05$).

2.7. Recording

Electroencephalographic recordings and behavioral observations were carried out in a glass compartment. After 15 min of habituation to the test cage, the basal electroencephalographic record was recorded for 15 min and the animals were then injected with the TsTX toxin solution preceded by the glutamate receptor antagonists. After the injections, continuous electroencephalographic recording and observations of animal behavior were performed for a period of 4 h. Fisher's test was used to analyze all the parameters observed ($P < .05$).

2.8. Histology

The correct location of the implanted deep electrodes and the cannulas or probes was checked histologically. The morphological analysis was performed 7 days after the injection. The animals were completely anesthetized with ether 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 1 week, and embedded in paraffin. 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 hippocampal area was analyzed by light microscopy using a 40 × magnification objective. A two-dimen-

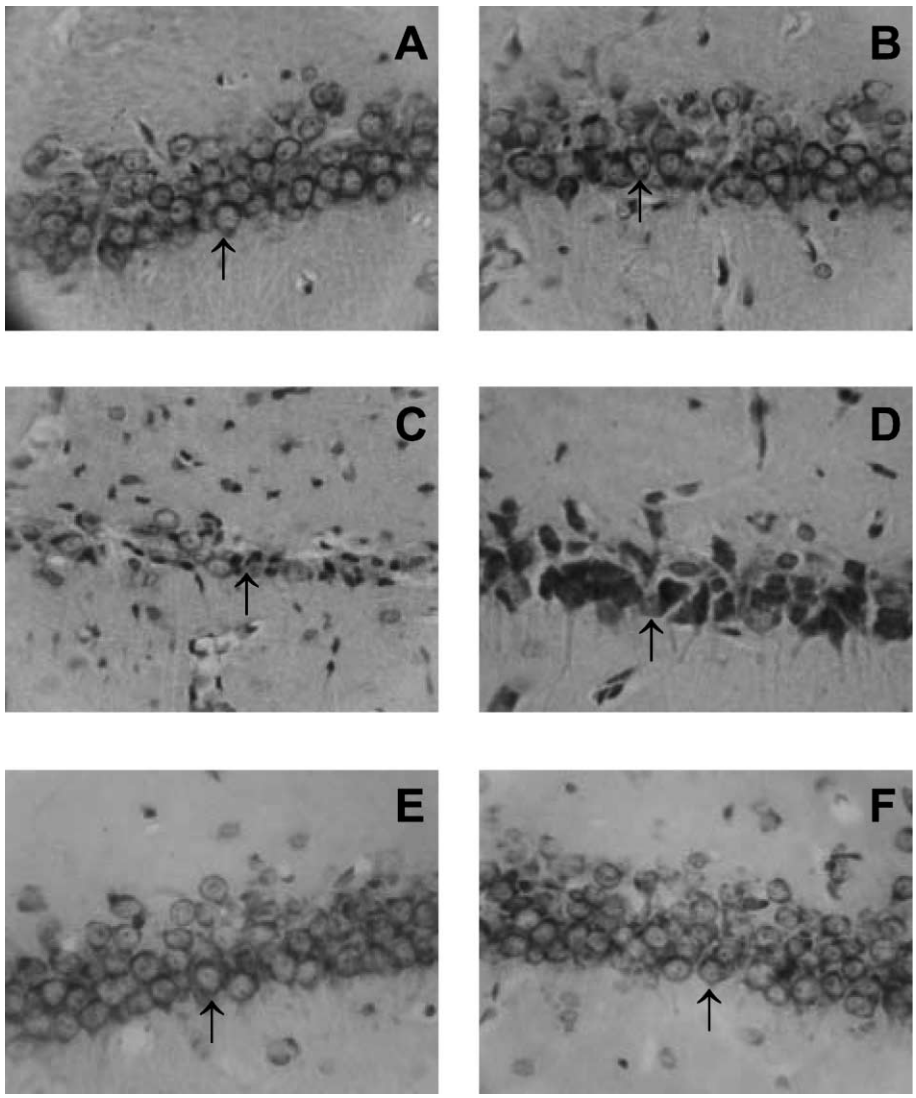

Fig. 4. Photomicrographs of the CA1 hippocampal area showing a typical pattern of neurodegeneration 7 days after microinjection. (A) Ringer's (ipsilateral) ($n = 4$); (B) Ringer's (contralateral) ($n = 4$); (C) 1.0 μg of TsTX toxin (ipsilateral) ($n = 8$); (D) 1.0 μg of TsTX toxin (contralateral) ($n = 8$); (E) 1.0 mg/kg of MK-801 and 1.0 μg/μl of TsTX toxin (ipsilateral) ($n = 5$); (F) 1.0 mg/kg of MK-801 and 1.0 μg/μl of TsTX toxin (contralateral) ($n = 5$). The control hippocampus appears to be unaffected (A, B). In (C) and (D) dark-staining neurons (pycnotic cells) are present throughout the entire section and in (E) and (F) MK-801 blocks the neurodegeneration evoked by the toxin. The arrows indicate the pyramidal cells. Cresyl violet stain. Magnification: 140 ×.

sional cell count was performed using a 100×100 μm reticulum. Only pyramidal neurons, localized inside the reticulum area, with a visible nucleus and nucleolus were considered intact. ANOVA followed by Tukey's test was used for statistical analysis ($P < .05$).

3. Results

3.1. Extracellular amino acid levels

The extracellular levels of glutamate were significantly increased in Samples 9, 11, and 12 when compared with the basal levels expressed in Samples 2, 3, and 4 (Fig. 1). Although the effect of the toxin is instantaneous, alterations in the extracellular glutamate levels was noted only after Sample 7 because the perfusate required 45 min to go through the outlet polyethylene tubing. No statistical alterations in extracellular GABA or glycine levels were observed (data not shown).

3.2. Electroencephalographic recordings and behavioral observations

Injection of Ringer's solution into the CA1 hippocampal area caused no alterations in behavior or in the electroencephalographic record (Table 1 and Fig. 2A). Injection of TsTX toxin solution induced long electroencephalographic epileptic-like discharges (Fig. 2B), immobility, orofacial movements, and "wet dog shakes" in all animals (Table 1). These manifestations started 5–10 min after the injection and persisted for the entire recording period. Injection of MK-801 induced agitation, postural loss, stereotypy and staggering ambulation (Table 1) and the electroencephalographic record showed clustered slow waves (Table 1 and Fig. 2D). These manifestations persisted for the entire recording period. Injection of MK-801 and TsTX blocked the epileptic discharges characteristic of the toxin (Table 1) although the animals conserved the behavioral alterations caused by the antagonist. No significant alterations in electroencephalographic activity or behavior were observed after injection of AP-5, CNQX, AP-3, or MCPG (Table 1). AP-5 injected before the toxin blocked the electrographic effects of TsTX, even though two animals (33.3%) showed isolated spikes (Fig. 2E) and wet dog shakes (Table 1). CNQX injected before the toxin prevented the appearance of epileptiform discharges in most rats (Table 1) but 33% of them had long discharges (Fig. 2B) followed by isolated spikes (Fig. 2E) and wet dog shakes that persisted for approximately 1 h after toxin injection (Table 1). Among the animals injected with AP, 28.5% ($n = 3$) had short discharges (Fig. 2C) and several wet dog shakes during the first hour of recording (Table 1) and the effects disappeared during the subsequent recording period. Only one animal (16.6%) injected with MCPG and TsTX had a short discharge (Fig. 2C) and wet dog shakes during a period of 45 min after injection of the toxin (Table 1). The others showed no alterations.

3.3. Histology

Analysis of the hippocampal sections after Ringer's solution injection showed no alterations in the number of cells (Figs. 3 and 4). TsTX injection showed neurodegeneration in CA1 pyramidal cell areas ipsi- and contralateral (contra) to the injection site (Figs. 3 and 4).

None of the antagonists alone caused cell loss in the CA1 area and when injected with TsTX they blocked neurodegeneration induced by the toxin (Figs. 3 and 4).

4. Discussion

Some works have demonstrated the releasing action of scorpion venom or purified toxins on the sympathetic and parasympathetic systems (Freire-Maia and Diniz, 1970; Freire-Maia and Campos, 1989; Freire-Maia et al., 1974; Couto et al., 1992). Coutinho-Netto et al. (1980) demonstrated that tityustoxin affected the release of other neurotransmitters such as GABA, aspartate, or glutamate in in vitro and in vivo preparations and Dorce and Sandoval (1994) demonstrated that whole venom enhanced dopamine turnover in the striatum and hypothalamus after intravenous and intrahippocampal injection. More recently, it was demonstrated that TsTX evokes glutamate release from cortical synaptosomes and calcium is involved in this release (Fletcher et al., 1996; Massensini et al., 1998).

Since intrahippocampal injection of TsTX causes effects similar to those induced by glutamate receptor stimulation (Carvalho et al., 1998), we postulated that glutamate is responsible for the neuropathological and behavioral response to TsTX. In an attempt to test our hypothesis, we determined extracellular levels of glutamate, GABA, and glycine after intrahippocampal injection of toxin.

Glutamate is considered to be the major excitatory neurotransmitter in the central nervous system and plays an important role in a wide variety of central functions (Scatton, 1994; Cunningham et al., 1994; Conn and Pin, 1997; Filliat et al., 1998). On the other hand, an excess of glutamate can induce pathological processes (Scatton, 1994; Knöpfel et al., 1995; Filliat et al., 1998). Pathways containing glutamate or related excitatory amino acids are definitely involved in the generation and propagation of epileptic seizures (Fisher, 1991; Cunningham et al., 1994) and mediate the death of central neurons in several human pathological conditions (Olney, 1986; Choi, 1988). GABA is the main inhibitory neurotransmitter in the central nervous system (Vornov, 1991), and glycine is both an inhibitory neurotransmitter in the medulla and an important co-agonist of the NMDA glutamate receptor (Leeson and Iversen, 1994).

Extracellular glutamate levels increased significantly after TsTX injection. The maximum release was obtained in

Sample 9 collected approximately 1 h after toxin injection when cerebral electrographic activity showed the highest epileptiform activity. This is in accordance with the hypothesis that TsTX increases excitatory amino acid release by acting on sodium channels, inhibiting their inactivation (Couraud et al., 1982). Massensini et al. (1998) reported that increasing concentrations of TsTX evoked a concentration-dependent glutamate release and intracellular concentration of sodium and calcium in synaptosomes. Also, elevated levels of glutamate were related to the epileptic threshold in the hippocampus and entorhinal cortex (Medina-Ceja et al., 2000). The origin of part of this released glutamate is probably exocytotic because Massensini et al. (1998) demonstrated that TsTX-induced release of glutamate in brain synaptosomes is Ca^{2+}-dependent. However, it was demonstrated that during ischemia adenosine triphosphate (ATP) is depleted and impairment of Na^+-K^+ ATPase results in a reduction in Na^+ ions and an increase in K^+ ions in the extracellular space. This causes inverse operation of the glutamate transporter and release of glutamate into the extracellular space (Yamaguchi et al., 1998; Rossi et al., 2000). TsTX slows Na^+ channel inactivation, decreasing extracellular Na^+ concentration (Fletcher et al., 1996; Cestèle and Catterall, 2000). Thus, the increase in extracellular glutamate may be due not only to exocytosis but also to a reversal of the Na^+-dependent glutamate transporter.

In order to confirm our hypothesis that toxin evokes enhanced glutamate release, rats were treated with ionotropic and metabotropic antagonists of glutamate receptors. All drug doses and injection times were chosen in accordance with their anticonvulsant and antineurotoxic activity based on literature data (Croucher et al., 1982; Vezzani et al., 1988; Riedel et al., 1996).

It is widely accepted that the NMDA subtype of glutamate receptor plays a prominent role in mechanisms of neuronal degeneration (Bagetta et al., 1990; Choi, 1994; Tortella and Hill, 1996) and in the initiation or spread of epileptic neuronal hyperactivity (Croucher et al., 1982; Lehmann et al., 1991). Competitive antagonists such as AP-5 and noncompetitive antagonists such as MK-801 of this receptor complex attenuate brain injury following brain insults (Hara et al., 1997), prevent the degeneration produced by administration of NMDA receptor agonists (Bagetta et al., 1990), and have an anticonvulsant action (Croucher et al., 1982; Meldrum, 1985; Dingledine et al., 1990). In the present study, MK-801 totally prevented neuronal damage and epileptic-like discharges but caused behavioral (agitation, stereotypy, and postural loss) and electrographic (a slower tracing) alterations commonly observed after its application (Popoli et al., 1997). AP-5 was also efficient in terms of behavioral, electrographic, and histologic protection.

Glutamate-activated biochemical and physiological processes are mediated not only by NMDA, but also via an activation of non-NMDA receptors. Many behavioral pharmacology assays indicate that AMPA receptors may modulate neuronal processes involved in phenomena such as electrically and chemically induced convulsions (Liljequist et al., 1995) and seem to be particularly important in mediating excitotoxicity (Bisaga et al., 1993). In our experiments, we observed that the AMPA receptor antagonist CNQX was partially efficient in blocking the neuronal discharges. This was probably due to an involvement of other excitatory amino acid receptor types in the convulsive effect. Thus, the blockade of only one type of receptor is not enough to reverse the process.

Studies on excitatory amino acid-mediated toxicity have suggested that the neurodegenerative mechanism is not restricted to any one receptor type but that different mechanisms of toxicity are involved (Maginn et al., 1995). The intrahippocampal injection of 1*S*,3*R*-ACPD, a Class I and II metabotropic glutamate receptor agonist (Tebano et al., 1997), produced a pattern of seizure and neuronal damage similar to that produced by NMDA (Sacaan and Schoepp, 1992), and agonists acting on Class I mGluRs act as convulsant in rodents (Conn and Pin, 1997). Riedel et al. (1996) examined the role of hippocampal mGluRs in hypoxia in rats in vivo. Histological analysis of hippocampal regions revealed attenuated neuronal cell loss in CA1 and CA3 after intraventricular injection of both L-AP3, a Class I mGluR antagonist (Conn and Pin, 1997), and MCPG, a Class I and II mGluR antagonist (Conn and Pin, 1997; Tebano et al., 1997). It was also demonstrated that mGluRs participate in the neurodegeneration seen following ischemia and in the neuroprotection of cells in the CA1 hippocampal region after AP-3 injection (Maginn et al., 1995). These data are in accordance with our results. Thus, the intrahippocampal injection of AP-3 and MCPG was efficient in the neuroprotection of the CA1 hippocampal area. The direct excitatory effects of mGluR activation on CA1 pyramidal cells are mediated by a Group I mGluR or a closely related receptor (Gereau IV and Conn, 1995). There is a high level of mGluR5 mRNA expressed in CA1 pyramidal cells (Abe et al., 1992; Testa et al., 1994). In contrast, mGluR1 is not present there and this may suggest that mGluR5 is a more likely candidate for an mGluR mediating the direct excitatory effects of mGluRs on CA1 pyramidal cells (Gereau IV and Conn, 1995). It was demonstrated that selective mGluR5 antagonists block seizures in a range of rodent models (Chapman et al., 2000). Tebano et al. (1997) suggested an involvement of Class II mGluRs in the genesis of in vitro epileptiform activity produced by some convulsants at hippocampal levels. This could explain why MCPG almost totally blocked the epileptiform activity produced by TsTX while AP-3, an antagonist of Class I receptors only, was not totally efficient in protecting from seizure activity.

All the antagonists used in the present study behaved as described in the literature when used to block or to reverse the effects of glutamate or its agonists. This fact reinforces the initial idea that the toxin used here acts by releasing mainly glutamate, although other neurotransmitters can be involved. The participation of these antagonists in the total or partial blockade of the toxin effects indicates the involvement of

several glutamate receptors in the generation of the convulsive/neurodegenerative effect.

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