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Behavioral, Electroencephalographic, and Histopathologic Effects of a Neuropeptide Isolated From *Tityus serrulatus* Scorpion Venom in Rats

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CARVALHO, F. F., A. L. A. NENCIONI, I. LEBRUN, V. A. C. DORCE AND M. R. L. SANDOVAL. *Behavioral, electroencephalographic, and histopathologic effects of a neuropeptide isolated from* Tityus serrulatus *scorpion venom in rats.* PHARMACOL BIOCHEM BEHAV **60**(1) 7–14, 1998.—The effects of intrahippocampal administration of a neuropeptide (TS-8F toxin) isolated from *Tityus serrulatus* scorpion venom have been determined on behavior, limbic seizures, and neuronal degeneration in rats. Behavioral observation showed orofacial automatism, wet dog shakes, and myoclonus. Concomitantly, the electroencephalographic record showed high-frequency and high-voltage spikes that evolved to seizure activity in the hippocampus and cortex. Seven days after TS-8F toxin microinjection, neuronal damage was observed in CA1 and CA2 pyramidal cells and in granular cells of the dentate gyrus. The results suggest that TS-8F toxin may be responsible, at least in part, by the epileptic effects observed with the crude venom. Thus, this toxin may be a useful tool in the study of some neurobiological process. © 1998 Elsevier Science Inc.

Scorpion Neurotoxin Seizure Neurodegeneration

VARIOUS toxins have been isolated from the venom of different scorpion species. From the venom of the Brazilian scorpion *Tityus serrulatus* the first one reported was tityustoxin (25), and the presence of others toxic components in the same venom was later demonstrated (36,39).

These neurotoxins have been classified into two main categories based on their known primary structure: short-chain toxins with less than 40 amino acids and long-chain toxins with 60–70 amino acids. They present specificity for Na^+ (5,7), K^+ (9), or Cl^- channels (18,31), being employed for the study of ionic channels (1,9,15).

Extensive research was carried out to describe the effects of scorpion venoms and their toxins on the metabolism (22), pancreas (36) and cardiovascular (3,41), neuromuscular (45), respiratory (2,3), renal (46), and gastrointestinal (16,17) systems. However, few reports are available about the effects of scorpion venom on the central nervous system (CNS). In this respect, it is known that convulsions are sometimes observed as clinical symptoms, mainly in children (8); *Centruroids sculpturatus* venom induced epileptiform waves when applied to the cerebral cortex of rats (1); the intracerebroventricular injection of *Leiurus quinquistriatus* or of *Tityus serrulatus* venom induced convulsion in rats (29,34). It was previously observed in our laboratory that a microinjection of *Tityus serrulatus* venom into the hippocampus induced an epileptiform pattern on the cerebral electric activity records of rats (40). On the other hand, Zhou et al. (47) have described an antiepileptic peptide purified from *Buthus martensii* venom. In this way, the present investigation was designed to study the behavioral, electroencephalographic, and histopathological effects of a peptide purified from *Tityus serrulatus* scorpion venom.

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METHOD

Purification

Venom. Freeze-dried fresh venom from the scorpion *Tityus serrulatus* was obtained from the Arthropod Laboratory of Butantan Institute.

Gel filtration chromatography. Sephadex G-50M (0.8 × 150 cm) and G-25F (0.6 × 120 cm) columns were equilibrated and the samples eluted with 0.02 M ammonium bicarbonate, pH 8.0, at room temperature (see legend to Fig. 1A, B, and C).

Reversed phase high-performance liquid chromatography (HPLC). The peptides purified by gel filtration chromatography were separated by HPLC using a C18M Bondapak column (Waters) with a 10–90% linear gradient of 0.1% trifluoroacetic acid and acetonitrile (90% in 0.1% trifluoroacetic acid) over a period of 20 min. The effluent was monitored by UV detection at 214 nm.

Amino acid sequence analysis. The amino acid sequence analysis of the purified scorpion peptide (TS-8F toxin) was performed with an Applied Biosystem Model 470A sequenator. The phenylthiohydantoin derivatives of the amino acids were identified with an Applied Biosystems Model 120A PTH-Analyzer.

Behavioral, Electroencephafographic, and Neurophatologic Studies

Animals. Male Wistar rats weighing 200–250 g were used. Upon their arrival to the laboratory (7 days before experiments), the rats were individually housed in wire mesh cages

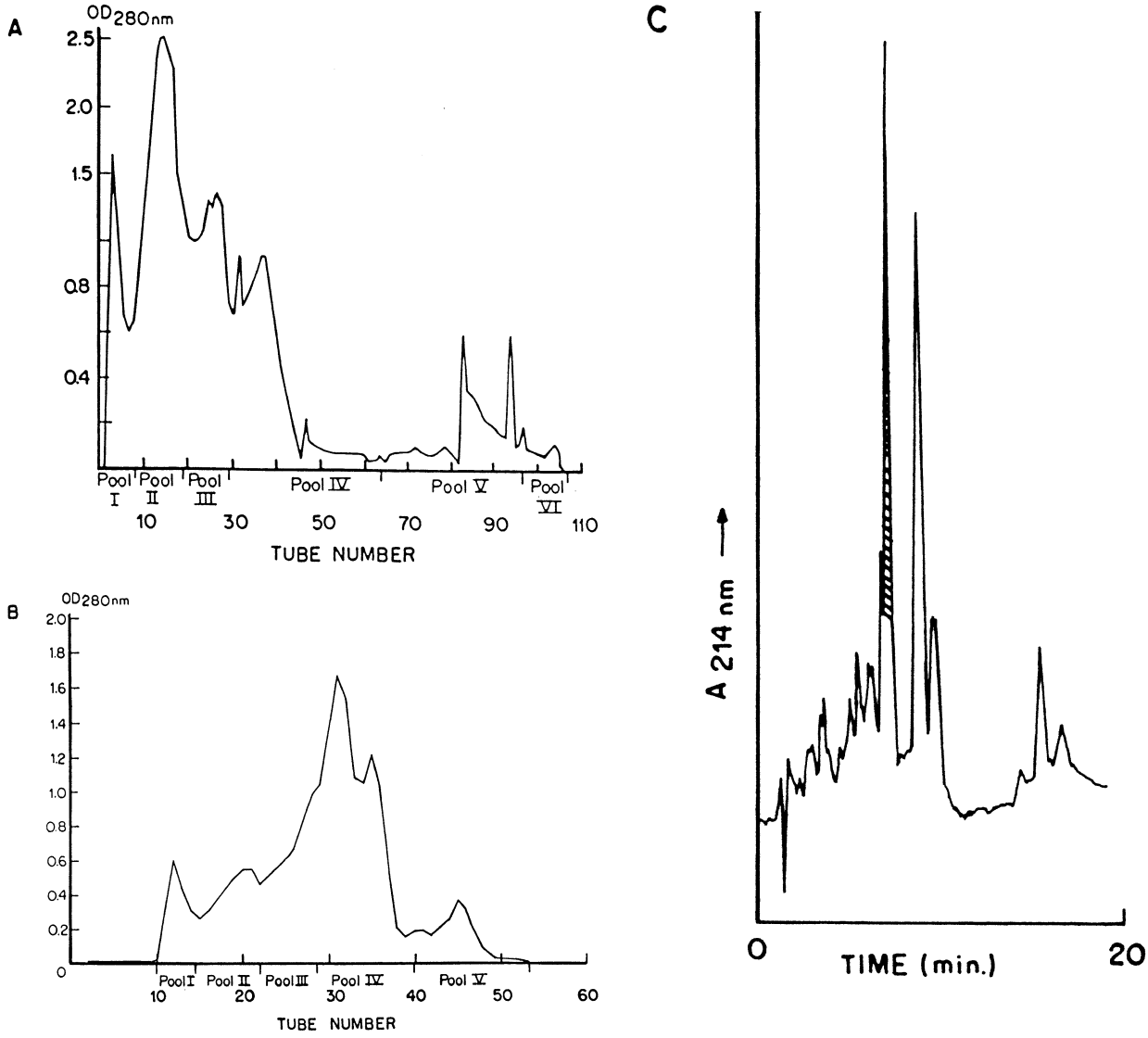

FIG. 1. (A) Sephadex G-50 M chromatography of *Tityus serrulatus* venom after extraction with 0.05 M ammonium bicarbonate buffer, pH 8.0, eluted with the same buffer (0.8 × 150 cm column): active fractions 30–63 Pool IV (B) Sephadex G-25 M column (0.6 × 120 cm) profile of the Sephadex G-50 active peak eluted with ammonium bicarbonate (0.05 M, pH 8.0): active fractions 28–39 Pool IV. (C) Analytical HPLC profile of the G-25 M active peak on a C 18 μBondapack column eluted with trifluoroacetic acid (0.1%) (solvent) and acetonitrile (90%) (solvent) and a solvent gradient developed from 10–90% of b within 20 min, monitored at 214 nm and 0.05 aufs (absorbance unit full scale). Peak 8 (hatched) showed significant activity in biological assays. OD, optical density.

and maintained in a room with constant temperature (22 ± 1°C), on a 12 L:12 D cycle (lights on at 0700 h); food and water were provided ad lib. The animals used in this study were maintained in accordance with the guidelines of the Department of Pathology at São Paulo University School of Veterinary Medicine following the guidelines for animal care prepared by the Committee on Care and Use of Laboratory Animal Resources, National Research Council, USA.

Surgery and recording. For electroencephalographic (EEG) records and cannula implantation rats were anesthetized (3 ml/kg) with a mixture of pentobarbitone (1 g) and chloralhydrate (4 g) in 100 ml of 0.9% NaCl, and positioned in a stereotaxic frame. For the injections, a guide cannula of stainless steel was chronically implanted and fixed with dental acrylate into one side of the dorsal hippocampus. For depth recordings, bipolar twisted electrodes were positioned in the dorsal hippocampus and anchored to the skull with dental acrylate. Coordinates were derived from the Atlas of Paxinos and Watson (35) (AP −4.8, L, 2.5, V 2.5 hippocampus). 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 5–7 days.

EEG recording and behavioral observations were carried out in a glass compartment. After 15 min of habituation to the test cage the basal EEG was recorded for 15 min and then the animals were microinjected into the hippocampal area with 1 μl of phosphate buffer or of TS-8F toxin solution (1 μg/μl or 2 μg/μl). After injection, EEG records and observation of the animal behaviors were made continuously until 2 h. After this time, EEG was not continuous, but a equivalent record time was performed for each animal until 6 h after injections and for periods of 30 min in the 3 subsequents days.

Morphological techniques. The correct location of the implanted deep electrodes and the cannulae was checked histologically. The morphological analysis was performed 7 days after the toxin injection. The animals were 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 and embedded in paraffin.

Coronal brain sections of 10 μm were cut from a 700 μm brain block including the cannula track. Every seventh slice for 300 μm from either side of the track was mounted on a glass slide and stained with cresyl violet.

RESULTS

Purification

Three hundred milligrams of crude *Tityus serrulatus* venom were extracted three times with ammonium bicarbonate buffer (pH 8.0) (1.0, 0.5, and 0.5 ml) and centrifuged at 5,000 rpm; the supernatant (2.0 ml) was applied to a Sephadex G-50 M column and the precipitate was discarded. The yield of the extraction process was 270 mg (OD 280). From the Sephadex G-50 M column 3.0 ml fractions were collected and six pools obtained according to OD values (Fig. 1A), the active pool IV was rechromatographed on a Sephadex G-25 F column and

TABLE 1

ELECTROGRAPHIC, BEHAVIORAL, AND HISTOPATHOLOGICAL EFFECTS OF 1 OR 2 μg OF Ts8F TOXIN MICROINJECTED IN DORSAL HIPPOCAMPUS IN INDIVIDUAL CASES

Case	Dose Ts8F	Frequency (0–2 h)		Duration (0–2 h)		Presence* (2–6 h)		Behaviors	Hippocampal Lesion
		Spikes	Seizures	Spikes	Seizures	Spikes	Seizures		
R1	1 μg	29	7	9.9	1.9	+++	++	Facial and corporal myoclonus, wet dog shake, immobility, rearing, circling behavior, orofacial movements	none
R2	1 μg	42	12	15.9	4.5	+	0	Wet dog shake, immobility	CA1, DG ipsilateral
R3	1 μg	6	0	4.1	0	0	0	Facial myoclonus, wet dog shake, rearing, liking, immobility	none
R4	1 μg	4	0	3.3	0	0	0	Facial myoclonus, wet dog shake	none
R5	1 μg	10	1	7.4	0.5	+	+	Facial myoclonus, wet dog shake, immobility, gnawing, sniffing	none
R6	1 μg	1	0	0.3	0	0	0	Facial myoclonus, wet dog shake	none
R7	2 μg	10	2	3.7	1.1	+++ +	++++	Orofacial movements, facial myoclonus, wet dog shake, wild running	CA1, CA2 ipsilateral
R8	2 μg	26	6	10.6	2.5	+++	++++	Facial and corporal myoclonus, postural loss, rearing, wet dog shake, circling behavior	CA1, CA2 ipsi and contralateral
R9	2 μg	18	25	14.4	9.7	+++	++	Wet dog shake, facial myoclonus, immobility	CA1 ipsilateral
R10	2 μg	12	4	3.9	1.1	+	++	Wet dog shake, facial myoclonus, immobility	CA1 ipsilateral
R11	2 μg	1	0	0.3	0	0	0	Facial myoclonus	none

The electrographic records were classified according to their pattern, every second of the tracing was classified as normal EEG, EEG with regular and irregular spikes or electrographic seizures. Each of these patterns is indicated as frequency or duration (in percent of 2 h record) of these episodes for each rat.

*Spikes or electrical seizures observed during the EEG records between 2–6 h. 0: no spikes, no seizures; +: 1–5 spikes or seizures; ++: 6–10 spikes or seizures; +++: 11–20 spikes or seizures; ++++: more than 20 spikes or seizures; +++++: seizure episodes longer than 20 min. After 2 h the EEG record was interrupted in different times.

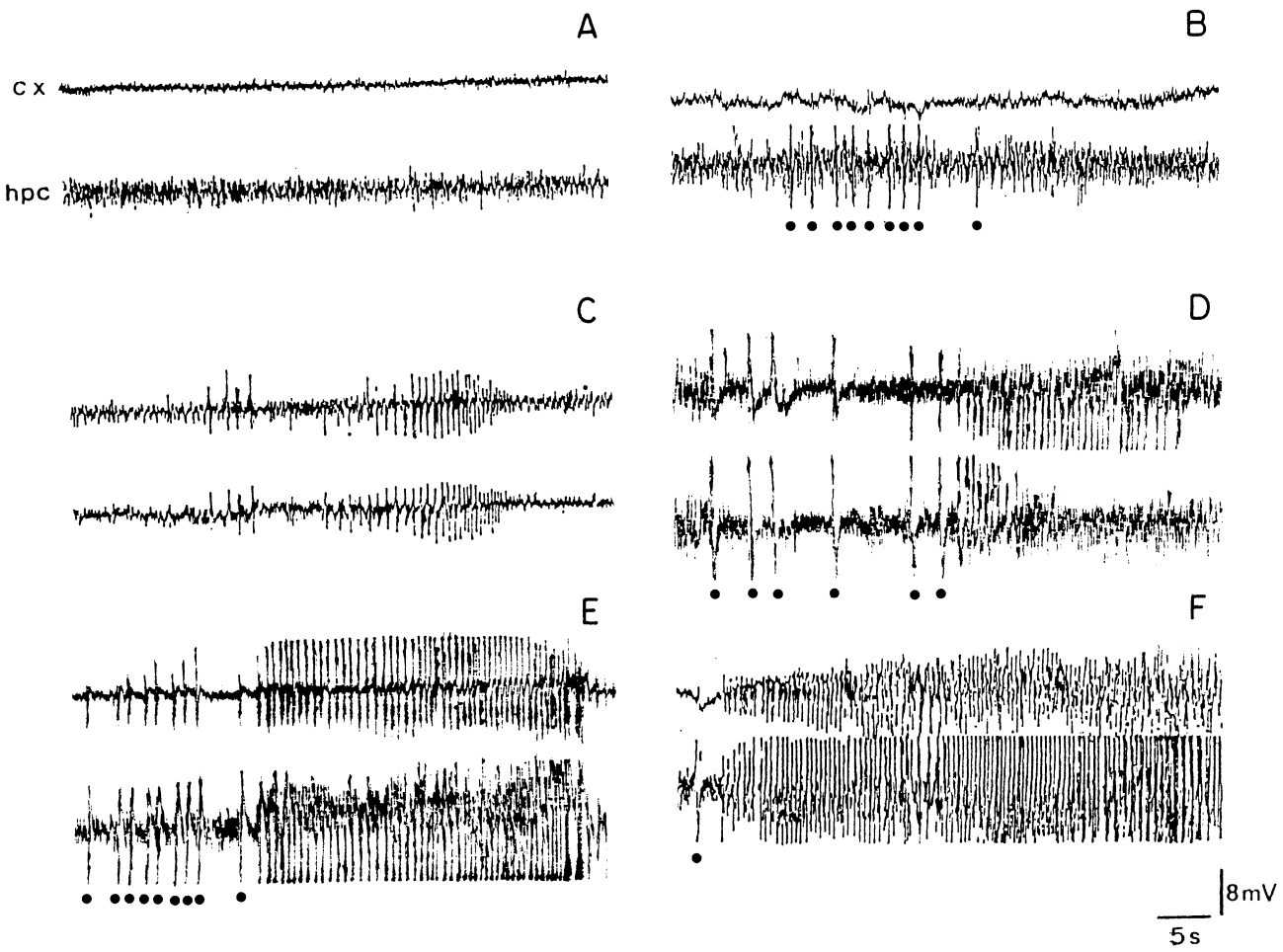

FIG. 2. Electroencephalographic records of a individual case (R7) to illustrate the alterations observed after TS-8F injection (2 μg) in the dorsal hippocampus. The records are from cortex (Cx) and hippocampus (hpc). (A) Control record before injection; (B) isolated spikes associated with wet dog shake (•); (C, D, E) symmetric and asymmetric burst of spikes, and (F) seizures observed repeatedly from 30 min to 6 h. The seizure discharges (F) were frequently accompanied by immobility.

the active fractions were pooled and lyophilized (Fig. 1B). The active pool was then applied to an HPLC apparatus resulting in 13 peaks (Fig. 1C). The active peak 8 (TS-8F toxin) was collected and lyophilized for amino acid sequence analysis and biological assays.

The amino acid analysis and N-terminal sequence showed the following composition:

Lys-Lys-Asp-Gly-Tyr-Pro-Val-Glu-Tyr-Asp-Asn-Cys-Ala-Tyr-Ile-Cys-Trp-Asn-Tyr-Asp-Asn-Ala-Tyr-Cys-Asp-Lys . . 10 20

This sequence is homologous to that described by Possani et al. (36) with 61 amino acid residues and 7077 MW and the full sequence is:

NH_2-Lys-Lys-Asp-Gly-Tyr-Pro-Val-Glu-Tyr-Asp-Asn-Cys-Ala-Tyr-Ile-Cys-Trp-Asn-Tyr-Asp-Asn-Ala-Tyr-Cys-Asp-Lys-Leu-Cys-Lys-Asp-Lys-Lys-Ala-Asp-Ser-Gly-Tyr-Cys-Tyr-Trp-Val-His-Ile-Leu-Cys-Tyr-Cys-Tyr-Gly Leu-Pro-Asp-Ser-Glu-Pro-Thr-Gly Ser-Thr-Lys-COOH

Behavioral, Electroencephalographic, and Neuropathologic Studies

Injection of TS-8F toxin solution (1 μg/μl; $n = 6$ or 2 μg/μl; $n = 5$) into the CA1 hippocampal area induced myoclonus and EEG epileptic-like discharges. The individual cases of treated rats have been reported in Table 1. Orofacial automatism, wet dog shakes, and myoclonus started to appear 5 to 10 min after the injection. At the same time, the EEG record showed initially high voltage fast activity with isolated spikes in the hippocampus (Fig. 2B). Within 30 min, spike activity and bursts of spikes started in the hippocampus, and in the cortex (Fig. 2C–E). The EEG activity evolved to seizure activity in hippocampus and cortex (Fig. 2F). No significant alterations were observed for frequencies of spikes or seizures and for duration of spikes or seizures (0–2 h) among the two utilized doses (Mann–Whitney U-test, $p < 0.05$). The observed electrographic and behavioral alterations persisted until 6 h, with greater intensity in rats injected with 2 μg of toxin and in the period ranging from 2 to 6 h. EEG alterations persisted for 48 h and were characterized by fast high-voltage activity with isolated spikes.

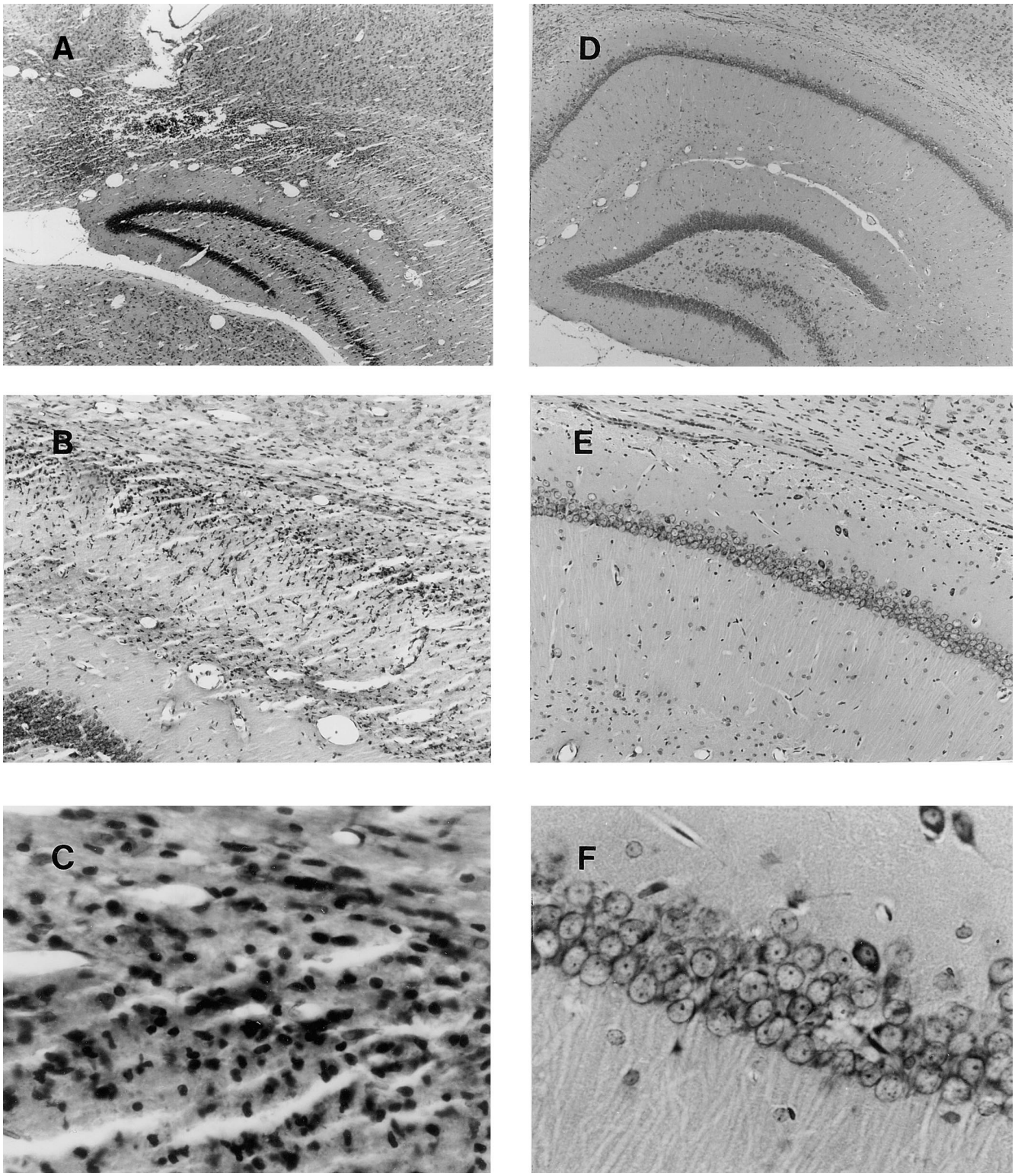

FIG. 3. Photomicrographs of the CA1 subfield of the hippocampus ipsilateral to the injection, showing a typical pattern of neurodegeneration 7 days after TS-8F toxin microinjection (A, B, C). Dark staining neurons are present throughout the entire section with few neurons appearing normal. The control hippocampus appears to be unaffected (D, E, F). Cresyl violet stain. ~Magnification: A, D ×15; B, E ×35; C, F ×140.

Seven days after the toxin injection, analysis of the hippocampal sections by light microscopy (Fig. 3) showed neurodegeneretion in CA1 (five animals) and CA2 (two animals) pyramidal cell areas and granular cells of the dentate gyrus (one animal) ipsilateral to the injection site. The lesions were manifested by massive cellular loss and gliosis and occurred mainly in those animals treated with 2 μg of toxin that developed intense electrographic seizure after the second hour of record. One animal showed neuronal loss on the contralateral side of the injection (CA1 and CA2 areas).

DISCUSSION

The isolated and sequenced TS-8F toxin showed full homology up to 26 amino acid residues with the IV-5 toxin isolated from *Tityus serrulatus* scorpion venom by Possani et al. (36). The previously described pharmacological characterization of this toxin was based only on its effect on guinea pig pancreatic secretion (36). Thus, the effects in the central nervous system characterizing neurotoxic and seizure actions are now being described for the first time.

The present results demonstrate that low doses of TS-8F toxin isolated from *Tityus serrulatus* scorpion venom microinjected into the dorsal hippocampus of rats induced behavioral alterations characterized by orofacial automatism, wet dog shakes, immobility, and myoclonus. EEG records exhibited an activity pattern that started with isolated spikes and evolved to epileptic discharges. Previously, we demonstrated that *Tityus serrulatus* crude venom microinjected into the hippocampus induced behavioral alterations similar to those now observed but accompanied by tonic–clonic seizure (40). Thus, it appears that, at least in part, the epileptic effects observed with crude venom can be elicited by the TS-8F toxin. The results obtained by Coutinho-Netto et al. (15) is in accordance with the present results. They observed that tityustoxin induced epileptic limb jerking that was abolished by tetrodotoxin.

It is well known that seizures produced by several convulsants are associated with excitotoxic effects (e.g., neuronal cell death) following systemic or intracerebral injection into different animals (27). In this way, kainic acid, an analog of the putative excitatory neurotransmitter glutamate, has a prominent toxic effect on hippocampus characterized by motor and electrographic convulsion and lesion of cell bodies (6,21). Also, α-dendrotoxin, a K^+ channel-blocking toxin isolated from a snake venom, that causes enhancement of excitability of synaptic transmission leads to motor and electrographic convulsions with brain damage when directly injected in hippocampus of rats (4). A single systemic injection of pilocarpine induces status epilepticus with hippocampal cell loss (43). The same is observed when crude venom of the snake *Crotalus durissus terrificus* was directly injected in hippocampus (33). In both cases the latency for the appearance of the first behavioral signs and the first convulsion are long (33,44), contrasting with the short latency for convulsions in several models of epilepsy including kainic acid and α-dendrotoxin models. In this way, TS-8F toxin now injected in hippocampus has a latency for the first electrographic convulsion similar to that described for *Crotalus durissus terrificus* venom (33). There is a similarity also between the behavioral signs observed in the three cases, namely episodes of immobility followed by several wet dog shakes and orofacial automatisms. These have been related to activation of limbic structures (33). It is interesting to note that Sacaan and Schoepp (38) described that a single intrahippocampal injection of 1S,3R-ACPD, a highly selective metabotropic of excitatory amino acids (EAA) receptor agonist, causes a delayed seizure-related behavioral syndrome characterized by akinesia, multiple episodes of wet dog shakes, jaw twitching, and moderate to severe limbic seizures.

The neurodegeneration induced by TS-8F toxin appears to be similar to that observed after intrahippocampal injection of *Crotalus durissus terrificus* venom (33), α-dendrotoxin (4), kainic acid (10), and after systemic administration of pilocarpine (43). These were characterized by cellular loss and gliosis. The present data suggest a similar mechanism for these effects with a possible involvement of metabotropic EAA receptor.

It is known that the scorpion toxins bind to neurotoxin receptor sites blocking sodium channel inactivation (14,30) or potassium channels (9,23,24). The binding to these channel receptors increases the depolarization time of the channel and consequently induces excessive neurotransmitter release. Experimentally scorpion toxins enhance the release of [U-^{14}C] glutamate and [U-^{14}C] GABA from rat preloaded cortical synaptosomes (15,20), of acetylcholine from rat brain (26) and of catecolamines from rat mioenteric plexus (42). Furthermore, tityustoxin caused a specific release of glutamate from cerebral synaptosomes of rats (37). The participation of the excitatory amino acid neurotransmitters in epilepsy, seizure-induced brain damage, and glutamate excitotoxicity has been extensively investigated but is not well understood (11,13, 19,28,32,38). It is known that the rat hippocampus has one of the highest densities of EAA receptors and is a brain structure that when electrically or chemically stimulated is able to induce convulsions and epileptiform discharges on the EEG recording.

In light of these facts, we suggest that the convulsive and neuronal loss effects induced by TS-8F toxin could be due to the enhanced release of EAA that are present in the most important hippocampal pathways (12).

There are several significant questions arising from these findings. Are EAA involved in the seizures and neurodegeneration induced by TS-8F toxin? Can the maintenance of the animal for long periods of time after TS-8F toxin microinjection lead to the development of spontaneous and/or recurrent seizures? And does brain damage persist, or does a regeneration or sprouting process occur?

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REFERENCES

1. Allon, N.; Woody, C. D.: Epileptic activity induced in single cells of the sensorimotor cortex of the cat by intracellularly applied scorpion venom. Exp. Neurol. 80:491–497; 1983.
2. Almeida, H. O.; Lima, E. G.; Freire-Maia, L.: Mechanism of the pulmonary edema induced by intracerebroventricular injection of scorpion toxin (tityustoxin) in the unanesthetized rat. Toxicon 14: 435–440; 1976.
3. Azevedo, A. D.; Silva, A. B.; Cunha-Melo, J. R.; Freire-Maia, L.:

Cardiovascular and respiratory effects induced by a purified scorpion toxin (tityustoxin) in unanesthetized rats. Toxicon 21:753–759; 1983.
4. Bagetta, G.; Nistico, G.; Jolly, J. O.: Production of seizures and brain damage in rats by α-dendrotoxin, a selective K^+ channel blocker. Neurosci. Lett. 139:34–40; 1992.
5. Barhanin, J.; Giglio, J. R.; Leopold, P.; Schmid, A.; Sampaio, S. V.; Lazdunski, M.: *Tityus serrulatus* venom contains two classes of toxins, tityus gamma-toxin is a new tool with a very high affinity for studying the Na^+ channel. J. Biol. Chem. 257:12553–12558; 1982.
6. Ben-Ari, Y.; Tremblay, E.; Ottersen, O. P.: Injections of kainic acid into the amygdaloid complex of the rat: An electrographic, clinical and histopathological study in relation to the pathology of epilepsy. Neuroscience 5:515–518; 1980.
7. Blaustein, M. P.; Rogowski, R. S.; Schneider, M. J.; Krueger, B. K.: Polypeptide toxins from the venoms of old world and new world scorpion preferentially block different potassium channels. Mol. Pharmacol. 40:932–942; 1991.
8. Campos, J. A.; Silva, O. S.; Lopes, M.; Freire-Maia, L.: Signs, symptoms and treatment of severe scorpion poisoning in children. In: Eaber, D.; Wadstrom, T., eds. Natural toxins. Oxford: Pergamon Press; 1980:61–68.
9. Carbone, E.; Wanke, E.; Prestipino, G.; Possani, L. D.; Maelicke, A.: Selective blockage of voltage-dependent K^+ channels by a novel scorpion toxin. Nature 296:90–91; 1982.
10. Cavalheiro, E. A.; Richie, D. A.; Le Gal La Salle, G.: Long-term effects of intrahippocampal kainic acid injection in rats: A method for inducing spontaneous recurrent seizures. Eletroencephalogr. Clin. Neurophysiol. 53:581–589; 1982.
11. Choi, D. W.: Glutamate neurotoxicity and diseases of the nervous system. Neuron 1:623–634; 1988.
12. Choi, D. W.: Glutamate neurotoxicity: A three-stage process. In: Guidotti, A., ed. Neurotoxicity of excitatory amino acids. New York: Raven Press; 1990:235–242.
13. Choi, D. W.: Glutamate receptors and the induction of excitotoxic neuronal death. Prog. Brain Res. 100:47–51; 1994.
14. Couraud, F.; Jover, E.; Dubois, J. M.; Rochat, H.: Two types of scorpion toxin receptor sites one related to the activation the other to the inactivation of the action potential sodium channel. Toxicon 20:9–16; 1982.
15. Coutinho-Netto, J.; Abdul-Ghani, A.-S.; Norris, P. J.; Thomas, A. J.; Bradford, H. F.: The effects of scorpion venom toxin on the release of amino acid neurotransmitters from cerebral cortex in vivo and in vitro. J. Neurochem. 35:558–565; 1980.
16. Cunha-Melo, J. R.; Almeida, A. P.; Gonzaga, H. M. S.; Gomez, M. V.; Freire-Maia, L.: Effect of scorpion toxin on gastric histamine and acethylcholine content in the rat. Braz. J. Med. Biol. Res. 20:393–401; 1987.
17. Cunha-Melo, J. R.; Toppa, N. H.; Martins, P.; Colares, C. N.; Castro, Y. R.; Freire-Maia, L.: Acute gastric mucosal injury induced by toxins from *Tityus serrulatus* scorpion venom: A novel experimental model in the rat. Toxicon 29:1395–1401; 1991.
18. DeBin, J. A.; Maggio, J. E.; Strichartz, G. R.: Purification and characterization of chlorotoxin a chloride channel ligand from the venom of the scorpion Am. J. Physiol. Soc. 264:C361–C369; 1993.
19. Dingledine, R.; Mcbain, C. J.; Mc Namara, J. D.: Excitatory amino acid receptors in epilepsy. Trends Pharmacol. Sci. 11:334–340; 1991.
20. Dolly, J. O.; Chun, K. Tse; Spokes, J. W.; Diniz, C. R.: Effects of β-Bungarotoxin and tityustoxin on uptake and release of neurotransmitters. Biochem. Soc. Trans. 6:652–654; 1978.
21. Fisher, R. S.: Animal models of epilepsies. In: Fisher, R. S.; Coyle, J. T., eds. Neurotransmitters and epilepsy. New York: Wiley-Liss, Inc.; 1991:61–76.
22. Freire-Maia, L; Ferreira, M. C.: Estudo do mecanismo da hiperglicemia e da hipertensão arterial, produzidas pelo veneno de escorpião no cão. Mem. Inst. Oswaldo Cruz. 59:11–12; 1961.
23. Garcia-Calvo, M.; Leonard, R. J.; Novick, J.; Stevens, S. P.; Schmalhofer, W.; Kaczorawski, G. J.; Garcia, M. L.: Purification, characterization, and biosynthesis of margatoxin, a component of Centruroides margaritatus venom that selectively inhibits voltage-dependent potassium channels. J. Biol. Chem. 268:18866–18874; 1993.
24. Gimenez-Gallego, G.; Navia, M. A.; Reuben, J. P.; Katz, G. M.; Kaczorowski, G. J.; Garcia, M. L.: Purification, sequence, and model structure of charybdotoxin, a potent selective inhibitor of calcium-actived potassium channels. Proc. Natl. Acad. Sci. USA 85:3329–3333; 1988.
25. Gomez, M. V.; Diniz, C. R.: Separation of toxic components from the Brazilian scorpion *Tityus serrulatus* venom. Mem. Inst. Butantan 33:899–902; 1966.
26. Gomez, M. V.; Dai, M. L.; Diniz, C. R.: Effect of scorpion venom, tityustoxin on the release of acetylcholine from incubated slices of rat brain. J. Neurochem. 20:1051–1061; 1973.
27. Griffiths, T.; Evans, M. C.; Meldrum, B. S.: Status epilepticus: The reversibility of calcium loading and acute neuronal pathologic changes in the rat hippocampus. Neuroscience 12:557–567; 1984.
28. Lambert, J. D. C.; Jones, R. S. G.; Andreasen, M.; Jensen, M. S.; Heinemann, U.: The role of excitatory amino acids in synaptic transmission in the hippocampus. Comp. Biochem. Physiol. 93A: 195–201; 1989.
29. Lima, E. G.; Almeida, H. O.; Gomez, M. V.; Freire-Maia, L.: Acute pulmonary edema induced by injection of tityustoxin into the lateral ventricle on rats. Toxicon 13:205–206; 1975.
30. Linden, C. D.; Raftery, M. A.: Isolation of a scorpion toxin for use as a probe of the electrically excitable sodium channel. Biochem. Biophys. Commun. 72:646–653; 1976.
31. Lippens, G.; Najib, J.; Wodak, S. J.; Tartar, A.: NMR sequential assignments and solution structure of chlorotoxin, a small scorpion toxin that blocks chloride channels. Biochemistry 34:13–21; 1995.
32. Meldrum, B.; Garthwaite, J.: Excitatory amino acid neurotoxicity and neurodegenerative disease. Trends Pharmacol. Sci. 11:379–387; 1990.
33. Mello, L. E. A. M.; Cavalheiro, E. A.: Behavioral, electroencephalographic and neuropathological effects of the intrahippocampal injection of the venom of the south American rattlesnake (*Crotalus durissus terrificus*). Toxicon 27:189–199; 1989.
34. Osman, O. H.; Ismail, M.; Wenger, T.: Hyperthermic response to intraventricular injection of scorpion venom: Role of brain monoamines. Toxicon 11:361–368; 1973.
35. Paxinos, G.; Watson, C.: The rat brain. New York: Academic Press; 1982.
36. Possani, L. D.; Martin, B. M.; Fletcher, M. D.; Fletcher, P. L., Jr.: Discharge effect on pancreatic exocrine secretion produced by toxins purified from *Tityus serrulatus* scorpion venom. J. Biol. Chem. 266:3178–3185; 1991.
37. Romano-Silva, M. A.; Gomez, M. V.; Brammer, M. J.: Modulation of Ca^+2-stimulated glutamate release from synaptossomes by Na^+ entry through tetrodotoxin-sensitive channels. Biochem. J. 304:353–357; 1994.
38. Sacaan, A. I.; Schoepp, D. D.: Activation of hippocampal metabotropic excitatory amino acid receptors leads to seizures and neuronal damage. Neurosci. Lett. 139:77–82; 1992.
39. Sampaio, S. V.; Laure, C. J.; Giglio, J. R.: Isolation and characterization of toxic proteins from the venom of the Brazilian scorpion *Tityus serrulatus*. Toxicon 21:265–277; 1983.
40. Sandoval, M. R. L. S.; Dorce, V. A. C.: Behavioral and electroencephalographic effects of *Tityus serrulatus* scorpion venom in rats. Toxicon 31:205–212; 1993.
41. Silveira, N. P.; Moraes-Santos, T.; Azevedo, A. D.; Freire-Maia, L.: Effects of *Tityus serrulatus* scorpion venom and one of its purified toxins (toxingamma) on the isolated guinea pig heart. Comp. Biochem. Physiol. 98C:329–336; 1991
42. Tafuri, W. L.; Freire-Maia, L.; Cunha-Melo, J. R.: Effect of purified scorpion venom on vesicular components in the myoenteric plexus of rat. Toxicon 8:427–430; 1971.
43. Turski, W. A.; Cavalheiro, E. A.; Schwarz, M.; Czuczwar, S. J.; Kleinrok, Z.; Turski, L.: Limbic seizures produced by pilocarpine in rats: Behavioral, electroencephalographic and neurophatological study. Behav. Brain Res. 9:315–336; 1983.
44. Turski, W. A.; Cavalheiro, E. A.; Bortolotto, Z. A.; Mello, L. E. A. M.; Schwarz, M.; Turski, L.: Seizures produced by pilocarpine in mice: A behavioral, electroencephalographic and morphological analysis. Brain Res. 321:237–253; 1984.
45. Vital Brazil, O.; Neder, A. C.; Corrado, A. P.: Effects and mecha-

nism of action of *Tityus serrulatus* venom on skeletal muscle. Pharmacol. Res Commun. 5:137–150; 1973.
46. Xavier, M. S.; Cardini, R. P.; Machado, R. P.; Alzamora, F.: Effect of phentolamine on the renal action of norepinephrine and scorpion venom. Braz. J. Med. Biol. Res. 19:536A; 1986.
47. Zhou, X.-H.; Yang, D.; Zhang, J.-H.; Liu, C.-M.; Lei, K.-J.: Purification and *N*-terminal partial sequence of anti-epilepsy peptide from venom of the scorpion Buthus martensis Karsch. Biochem. J. 257:509–517; 1989.