ELSEVIER

Comparative Biochemistry and Physiology, Part A 140 (2005) 125–134

CBP

www.elsevier.com/locate/cbpa

Biological and enzymatic activities of *Micrurus* sp. (Coral) snake venoms

Alessandra L. Cecchini[a,b], Silvana Marcussi[c,f], Lucas B. Silveira[c], Caroline R. Borja-Oliveira[d], Léa Rodrigues-Simioni[d], Susan Amara[b], Rodrigo G. Stábeli[e], José R. Giglio[f], Eliane C. Arantes[a], Andreimar M. Soares[c,g,*]

[a]*Departamento de Física e Química, FCFRP, USP, Ribeirão Preto-SP, Brazil*
[b]*Howard Hughes Medical Institute-Reseach Laboratories. Oregon Health Sciences University-Vollum Institute, Sam Jackson Park Road Portland, USA*
[c]*Unidade de Biotecnologia, Universidade de Ribeirão Preto, UNAERP, Ribeirão Preto-SP, Brazil*
[d]*Departamento de Farmacologia, UNICAMP, Campinas-SP, Brazil*
[e]*Laboratório de Bioquímica e Biotecnologia, Instituto de Pesquisas em Patologias Tropicais (IPEPATRO), Porto Velho-RO, Brazil*
[f]*Departamento de Bioquímica e Imunologia, FMRP, USP, Ribeirão Preto-SP, Brazil*
[g]*Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, FCFRP, USP, Ribeirão Preto-SP, Brazil*

Received 28 May 2004; received in revised form 11 November 2004; accepted 15 November 2004

Abstract

The venoms of *Micrurus lemniscatus carvalhoi*, *Micrurus frontalis frontalis*, *Micrurus surinamensis surinamensis* and *Micrurus nigrocinctus nigrocinctus* were assayed for biological activities. Although showing similar liposome disrupting and myotoxic activities, *M. frontalis frontalis* and *M. nigrocinctus nigrocinctus* displayed higher anticoagulant and phospholipase A_2 (PLA_2) activities. The latter induced a higher edema response within 30 min. Both venoms were the most toxic as well. In the isolated chick biventer cervicis preparation, *M. lemniscatus carvalhoi* venom blocked the indirectly elicited twitch-tension response (85±0.6% inhibition after a 15 min incubation at 5 μg of venom/mL) and the response to acetylcholine (ACh; 55 or 110 μM), without affecting the response to KCl (13.4 mM). In mouse phrenic nerve-diaphragm preparation, the venom (5 μg/mL) produced a complete inhibition of the indirectly elicited contractile response after 50 min incubation and did not affect the contractions elicited by direct stimulation. *M. lemniscatus carvalhoi* inhibited ^{3}H-L-glutamate uptake in brain synaptosomes in a Ca^{2+}, but not time, dependent manner. The replacement of Ca^{2+} by Sr^{2+} and ethylene glycol-bis(β-aminoethyl ether) (EGTA), or alkylation of the venom with *p*-bromophenacyl bromide (BPB), inhibited ^{3}H-L-glutamate uptake. *M. lemniscatus carvalhoi* venom cross-reacted with postsynaptic α-neurotoxins short-chain (antineurotoxin-II) and long-chain (antibungarotoxin) antibodies. It also cross-reacted with antimyotoxic PLA_2 antibodies from *M. nigrocinctus nigrocinctus* (antinigroxin). Our results point to the need of catalytic activity for these venoms to exert their neurotoxic activity efficiently and to their components as attractive tools for the study of molecular targets on cell membranes.

Keywords: Coral snake venoms; *Micrurus* sp.; *Micrurus lemniscatus carvalhoi*; Liposome-disrupting activity; Myotoxicity; Neurotoxicity; Phospholipase A_2; Synaptosome; L-glutamate uptake

Abbreviations: anti-BGTX, antibodies against bungarotoxin from *Bungarus multicinctus*; anti-NGX, antibodies against nigroxin from *Micrurus nigrocinctus nigrocinctus*; anti-NT-I, antibodies against neurotoxin I from *Naja naja oxiana*; anti-NT-II, antibodies against neurotoxin II from *Naja naja oxiana*; BPB, *p*-bromophenacyl bromide; CK, creatine kinase; $DIBAC_4(3)$, bis[1,3-dibutylbarbituric acid-(5)] trimethin-eoxolnol; DMEM, Dulbecco's modified eagle medium; EGTA, ethylene glycol-bis(β-aminoethyl ether); GABA, γ-aminobutyric acid; i.c.v., intracerebroventricular; PBS, phosphate buffered saline; PLA_2, phospholipase A_2; RCCS, rat cortico-cerebral synaptosomes; TTX, tetrodotoxin; VDCC, voltage-dependent Ca^{++} channel; VDSC, voltage-dependent Na^+ channel.

* Corresponding author. Tel.: +55 16 603 6892; fax: +55 16 603 7030.
E-mail address: andreimar@unaerp.br (A.M. Soares).

1095-6433/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpb.2004.11.012

1. Introduction

Coral snakes comprise a group of almost 50 species from the genus *Micrurus* found in the Southern United States and South America. They are a taxonomic assembly of more than 120 species and subspecies, achieving their greatest diversity near the equator (Rose and Bernal-Carlo, 1987). However, the mode of action of the venom of only a few species has been investigated.

The signs and symptoms of envenomation by *Micrurus* sp. are the result of a progressive blockade at the neuromuscular endplate and, in severe cases, death results from respiratory arrest. Besides supportive clinical care, serotherapy with heterologous antivenoms is the only treatment for coral snake bite envenomation (Russel, 1983; Bolaños, 1984). Experimental studies suggest the presence of a considerable spectrum of pharmacological activities of *Micrurus* venoms. They induce neurophysiological changes similar to those induced by α-neurotoxins, and some of them also show postsynaptic effects (Goularte et al., 1983; Vital-Brazil, 1987).

Some *Micrurus* venoms have been characterized according to their neurotoxic activities. *Micrurus corallinus* venom was described as having both presynaptic and postsynaptic actions. *M. corallinus* venom produces an irreversible neuromuscular blockade, reducing evoked acetylcholine (ACh) release and increasing the spontaneous release of ACh. *M. lemniscatus* and *M. frontalis* venoms demonstrated only a postsynaptic action (Vital-Brazil, 1987).

Micrurus venoms also showed myotoxicity (Gutiérrez et al., 1980, 1983, 1986, 1992) and cardiotoxicity when injected intravenously (Ramsey et al., 1972). Common characteristics as well as variability in some biological activities among venoms from different *Micrurus* species have been demonstrated in comparative studies (Gutiérrez et al., 1983, 1992; Aird and Da Silva, 1991; Tan and Ponnudurai, 1992; Alape-Girón et al., 1994).

Electrophoretic, immunochemical and chromatographic studies of *Micrurus* venoms have shown the presence of components with profile similar to several toxins from other elapids (Jorge-Da-Silva et al., 1991; Alape-Girón et al., 1994, 1996, 1999). Almost all *Micrurus* venoms have a high enzymatic phospholipase A_2 (PLA_2) activity but different profiles for other enzymes (Aird and Da Silva, 1991; Tan and Ponnudurai, 1992). For example, some of these venoms have anticholinesterase and anticoagulant activities in vitro (Kumar et al., 1973; Tan and Ponnudurai, 1992; Alape-Girón et al., 1996).

Since knowledge on the mechanism of action of this venom may be helpful in establishing protocols for the treatment of persons envenomed by this species, we have investigated the enzymatic and pharmacological effects evoked by the venom from four coral snakes, especially focusing on the neurotoxicity and inhibition of ^{3}H-L-glutamate uptake induced by *Micrurus lemniscatus carvalhoi* venom.

2. Materials and methods

2.1. Materials

The venoms from *Micrurus* sp. (*M. lemniscatus carvalhoi*, *Micrurus frontalis frontalis* and *Micrurus surinamensis surinamensis*) were kindly supplied by Luiz H. Anzaloni-Pedrosa, FMRP, USP, Brazil. *Micrurus nigrocinctus nigrocinctus* venom and antineurotoxins antibodies were kindly provided by Dr. Alberto Alape-Girón (Instituto Clodomiro Picado, Universidad de Costa Rica, San Jose, Costa Rica). ^{3}H-L-Glutamate was purchased from Perkin-Elmer Life Science; ScintiVerse, OptiPhase Supermix and the 1900 TR Liquid Scintillation Analyser were from Fisher Scientific, Wallac and Packard, respectively. BRANDEL system—Biomedical Research Development Laboratories, Gaithersburg, MD USA. All other reagents used were purchased from Sigma-Aldrich and Mallinckrodt.

2.2. Enzymatic and anticoagulant activities

Micrurus venom PLA_2 activity was evaluated using egg yolk as substrate (de Haas et al., 1968). Anticoagulant activity was assessed as described earlier (Alvarado and Gutiérrez, 1988).

2.3. Myotoxic activity

The assay of plasma creatine kinase (CK) activity was carried out using the CK-UV kinetic kit from Sigma. Venoms (5 μg) were injected intramuscularly in the gastrocnemius muscle of 18–22 g male Swiss mice (50 μL, n=6). Animals used as negative controls were injected with phosphate buffered saline (PBS). After 3 h, a blood sample was collected from the tail in heparinized capillary tubes and centrifuged for plasma separation (Soares et al., 2000a,b). The enzyme activity was expressed in U/L, one unit producing 1 μmol of NADH/min under the conditions of the assay.

2.4. Edema-inducing activity

Groups of five male Swiss mice (18–22 g) were injected subcutaneously in the subplantar region with 50 μL of venom (3.5 μg). At different intervals, the thickness of the paw was measured with a low-pressure spring caliper (Mitutoyo, Japan) as an index of edema (Soares et al., 2000a,b). Zero time values were subtracted from the corresponding final values, and the differences were expressed as percentage increment.