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ADP is a vasodilator component from *Lasiodora* sp. mygalomorph spider venom☆

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ABSTRACT

Members of the spider genus *Lasiodora* are widely distributed in Brazil, where they are commonly known as caranguejeiras. *Lasiodora* spider venom is slightly harmful to humans. The bite of this spider causes local pain, edema and erythema. However, *Lasiodora* sp. spider venom may be a source of important pharmacological tools. Our research group has described previously that *Lasiodora* sp. venom produces bradycardia in the isolated rat heart. In the present work, we sought to evaluate the vascular effect of *Lasiodora* sp. venom and to isolate the vasoactive compounds from the venom. The results showed that *Lasiodora* spider venom induced a concentration-dependent vasodilation in rat aortic rings, which was dependent on the presence of a functional endothelium and abolished by the nitric oxide synthase (NOS) inhibitor L-NAME. Western blot experiments revealed that the venom also increased endothelial NOS function by increasing phosphorylation of the Ser^{1177} residue. Assay-directed fractionation isolated a vasoactive fraction from *Lasiodora* sp. venom. Mass spectrometry (MS) and nuclear magnetic resonance (NMR) assays identified a mixture of two compounds: adenosine diphosphate (ADP, approximately 90%) and adenosine monophosphate (AMP, approximately 10%). The vasodilator effects of *Lasiodora* sp. whole venom, as well as ADP, were significantly inhibited by suramin, which is a purinergic P2-receptor antagonist. Therefore, the results of the present work indicate that ADP is a main vasodilator component of *Lasiodora* sp. spider venom.

1. Introduction

Spider venoms are a complex mixture of substances, combinatorial libraries of molecules, which act on various physiological targets. These bioactive compounds are important tools with applications in basic research, as well as in medicine, as potential drugs for the treatment of pain,

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diabetes, multiple sclerosis and cardiovascular diseases (Harvey et al., 1998; Lewis and Garcia, 2003; Bogin, 2005; Escoubas, 2006; Rates et al., 2011).

In general, venoms have three main components, including low molecular mass organic molecules (M_r < 1000 Da), polypeptides (M_r 3000–10,000 Da), and high molecular mass proteins (M_r > 10,000 Da) (Escoubas et al., 2000; Sollod et al., 2005). Although the major toxins of spider venoms are neurotoxic peptides, which act on a vast array of ion channels (Kushmerick et al., 1999; Escoubas et al., 2000; Gomez et al., 2002; Matavel et al., 2002; King and Hardy, 2013), non-neurotoxic peptides and also non-peptidic molecules have been described (Bento et al., 1993; Marangoni et al., 1993; Rego et al., 1996; Rash and Hodgson, 2002).

Lasiodora spiders are members of Theraphosidae family (suborder Mygalomorphae). They are commonly known in Brazil as caranguejeiras. The different species of *Lasiodora* spiders are difficult to distinguish (Brazil and Vellard, 1926). These spiders, as predators, use their venom to feed on a variety of both vertebrate and invertebrate prey. Moreover, the ability to paralyze higher vertebrates makes the venoms of all mygalomorph spiders intriguing sources of compounds for the study of various receptors in vertebrates (Escoubas and Rash, 2004).

Reports on bites in humans caused by mygalomorph spiders are rare. The clinical symptoms observed after the bite are local pain, edema and erythema (Lucas et al., 1994; Isbister et al., 2003). *Lasiodora* sp. spider venom has not been systematically studied. However, even venoms with low human toxicity can be sources for interesting physiological research (Escoubas and Rash, 2004).

We have previously described that *Lasiodora* sp. crude venom inhibits L-type Ca^{2+} channels (Ca_v1) and modulates the activity of Na^+ channel in GH3 cells (Kushmerick et al., 2001). Vieira et al. (2004) identified three toxins expressed by the *Lasiodora* sp. venom gland. These toxins, named LTx1, LTx2 and LTx3, showed significant similarity with other toxins from *Lasiodora parahybana*, *Eurypelma californicum*, *Brachypelma smithii* and *Selenocosmia huwena* spider venoms. Dutra et al. (2008) observed that recombinant LTx2 blocks Ca_v1 channels in BC3H1 cells.

Our research group has also described the action of *Lasiodora* sp. venom on the isolated rat heart. This venom caused concentration-dependent bradycardia, with transient cardiac arrest and rhythm disturbances. Therefore, the authors suggested that *Lasiodora* crude venom evokes vesicular release of acetylcholine from parasympathetic nerve terminals by activating tetrodotoxin-resistant Na^+ channels (Kalapothakis et al., 2003).

Thus, *Lasiodora* sp. venom may be a potential source of active toxins in various physiological systems, including the cardiovascular system. Many venom components, including bradykinin-potentiating peptides, sarafotoxins, and natriuretic peptides have significant cardiovascular effects (Hodgson and Isbister, 2009; Camargo et al., 2012).

The aim of the present work was to characterize the pharmacological action of *Lasiodora* sp. venom on rat aortic rings, as well as to isolate the vasoactive components from the venom. In this study, we report for the first time the vasodilator activity of *Lasiodora* sp. venom, which is dependent on endothelial nitric oxide (NO). Furthermore, we used assay-directed fractionation protocols, mass spectrometry (MS) and nuclear magnetic resonance (NMR) analysis to isolate and identify one main vasoactive molecule from *Lasiodora* sp. venom: adenosine diphosphate (ADP).

2. Material and methods

2.1. Drugs

The drugs used were all purchased from Sigma–Aldrich (St. Louis, MO, USA). Indomethacin was dissolved in 0.5% w/v sodium bicarbonate. The other compounds were dissolved in distilled water. For isolated aorta protocols, drugs were diluted in Krebs–Henseleit solution before the experiments.

2.2. Spiders and venom

Lasiodora specimens were from the city of Uberlândia in the state of Minas Gerais, Brazil. A voucher specimen of the spider under study has been deposited as collection number IBSP 8539 in the Instituto Butantan, located in São Paulo, Brazil. *Lasiodora* venom was obtained by electrical shock of the chelicerae using a custom stimulator, which included a guard to avoid contamination of the venom by regurgitated stomach contents. After extraction, the venom was stored immediately at −20 °C. Protein concentration in the venom was measured as described by Bradford (1976).

2.3. Experimental animals

Male Wistar rats (210–300 g) from the Animal Care facilities (CEBIO) at the Federal University of Minas Gerais (UFMG) were used. They were kept at 22–25 °C in a 12 h light/dark cycle, and had free access to food and water. Animal experiments were performed according to the recommendations of the Brazilian Council for Animal Care and were approved by the Ethics Committee (protocols 166/07 and 234/12 CETEA) of UFMG.

2.4. Rat aortic rings preparation and mounting

This protocol was performed as described by Cruz et al. (2006). Male Wistar rats were decapitated and exsanguinated. The descending thoracic aorta was excised, free of fat and connective tissue, cut into rings about 4–5 mm in length and set up in an organ chamber containing Krebs–Henseleit solution [(mM): NaCl, 110.8; KCl, 5.9; $NaHCO_3$, 25.0; $MgSO_4$, 1.07; $CaCl_2$, 2.49; NaH_2PO_4, 2.33; glucose, 11.51]. When necessary, the endothelium was removed mechanically by gently rubbing the intimal surface. The tissues were constantly gassed with a carbogenic mixture (95% O_2 and 5% CO_2), maintained at 37 °C under a tension of 1 g, and equilibrated for 1 h before initiating experimental protocols. During this period, the incubation solution was changed every 15 min. After the equilibration period, the presence of functional endothelium was assessed by the ability of acetylcholine (10 μM) to induce more than 80% relaxation of vessels pre-contracted with phenylephrine