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Pharmacological Characterization of the Edema Caused by *Vitalius dubius* (Theraphosidae, Mygalomorphae) Spider Venom in Rats

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ABSTRACT

Bites by tarantulas (Theraphosidae, Mygalomorphae) in humans can result in mild clinical manifestations such as local pain, erythema, and edema. *Vitalius dubius* is a medium-sized, nonaggressive theraphosid found in southeastern Brazil. In this work, we investigated the mediators involved in the plasma extravasation caused by *V. dubius* venom in rats. The venom caused dose-dependent (0.1–100 μg/site) edema in rat dorsal skin. This edema was significantly inhibited by ((S)1-{2-[3(3-4-dichlorophenyl)-1-(3-iso-propoxyphenylacetyl)piperidine-3-yl] ethyl}-4-phenyl-1-azoniabicyclo[2.2.2]octone, chloride) (SR140333, a neurokinin NK_1 receptor antagonist), indomethacin [a nonselective cyclooxygenase (COX) inhibitor], cyproheptadine (a serotonin 5-hydroxytryptamine$_{1/2}$ and histamine H_1 receptor antagonist), and N$^{\omega}$-nitro-L-arginine methyl ester (a nitric oxide synthase inhibitor). In contrast, mepyramine (a histamine H_1 receptor antagonist), D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8-]-BK (JE 049, a bradykinin B_2 receptor antagonist), and ((S)-N-methyl-N-[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-di-chlorophenyl)-butyl]benzamide) (SR48968, a neurokinin NK_2 receptor antagonist) had no effect on the venom-induced increase in vascular permeability. In rat hind paws, the venom-induced edema was attenuated by ketoprofen (a nonselective COX inhibitor) administered 15 minutes postvenom. Preincubation of venom with commercial antiarachnid antivenom attenuated the venom-induced edema. These results suggest that the enhanced vascular permeability evoked by *V. dubius* venom involves serotonin, COX products, neurokinin NK_1 receptors, and nitric oxide formation. The attenuation of hind paw edema by ketoprofen suggests that COX inhibitors could be useful in treating the local inflammatory response to bites by these spiders.

Introduction

Spiders of the family Theraphosidae (suborder Mygalomorphae), popularly known as tarantulas, have a widespread distribution throughout the tropics. The venoms of these spiders contain a variety of toxins that target primarily ion channels (Escoubas, 2006; Estrada et al., 2007; Herzig et al., 2011). Despite their large size and intimidating appearance, most theraphosids show low aggressivity, and this has led to their popularity as pets, particularly in North America and Europe. Although theraphosids have potentially large venom yields compared with other spiders, the clinical manifestations of human envenoming by these spiders are generally limited to local effects that include varying degrees of pain, erythema, and edema (Lucas et al., 1994; Isbister et al., 2003); however, prolonged muscle cramps have been reported after envenoming by *Poecilotheria regalis* (Indian ornamental tree spider) (Fuchs et al., 2014). Many theraphosid species are also capable of releasing urticating hairs that cause an allergic reaction in sensitive individuals (Battisti et al., 2011). Experimental studies in vertebrates (Bücherl, 1971; Bettini and Brignoli, 1978; Atkinson, 1993), together with the findings of clinical reports of envenoming in humans, indicate that the effects of theraphosid venoms are more severe in domestic animals and may include death (Robinson and Griffin, 1985; Raven, 2000; Isbister et al., 2003).

The theraphosid genus *Vitalius* contains at least nine species found predominantly in southeastern Brazil, with *Vitalius dubius* occurring in the southern part of the Brazilian state of Minas Gerais and in the state of São Paulo (Bertani, 2001). We have previously reported on the venom gland structure (Rocha-e-Silva et al., 2009a) and general venom composition (Rocha-e-Silva et al., 2009b) of *V. dubius*. We have also isolated a nicotinic receptor antagonist (Rocha-e-Silva et al., 2013) and hyaluronidase (Sutti et al., 2014) from this venom. In this work, we have extended our investigation of *V. dubius* venom by examining its ability to increase vascular permeability in rat dorsal skin and hind paw. We also assessed some of the mediators involved and examined the ability of

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ABBREVIATIONS: COX, cyclooxygenase; L-NAME, N$^{\omega}$-nitro-L-arginine methyl ester.

postvenom treatment with ketoprofen, a nonselective cyclooxygenase (COX) inhibitor, to attenuate venom-induced hind paw edema.

Materials and Methods

Animals. Male Wistar-Hanover rats (200–250 g) were obtained from the Multidisciplinary Center for Biologic Investigation (CEMIB/Universidade Estadual de Campinas) and were housed 5/cage at 23°C on a 12-hour light/dark cycle, with free access to food and water. The experimental protocols were approved by an institutional Committee for Ethics in Animal Use (CEUA/Universidade Estadual de Campinas, protocol 1228-1 and CEUA/Santa Casa, protocol 001/13), and the general ethical guidelines for animal use established by the Brazilian Society of Laboratory Animal Science (SBCAL) and EC Directive 86/609/EEC for animal experiments were followed.

Drugs. Bradykinin, cyproheptadine, compound 48/80, histamine, indomethacin, mepyramine, N^{ω}-nitro-L-arginine methyl ester (L-NAME), and Evan's blue dye were purchased from Sigma-Aldrich (St. Louis, MO). Ketoprofen [(RS)2-(3-benzoylphenyl)-propionic acid] was purchased from Sanofi-Aventis Farmacêutica (Susano, SP, Brazil). SR140333 ((S)1-{2-[3(3-4-dichlorophenyl)-1-(3-iso-propoxyphenylacetyl) piperidine-3-yl]ethyl}-4-phenyl-1-azoniabicyclo[2.2.2]octone, chloride) and SR48968 ((S)-N-methyl-N-[4-(4- acetylamino-4-phenylpiperidino)-2-(3,4-di-chlorophenyl)butyl]benzamide) were a gift of Dr. Xavier Emonds-Alt, (Sano Recherche, Montpellier, France). JE 049 (D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8-]-BK) was a gift of Hoechst AG (Frankfurt, Germany). ^{125}I-labeled human serum albumin was obtained from the Institute for Energy and Nuclear Research/National Commission for Nuclear Energy, University of Saõ Paulo (IPEN/CNEN-USP). Isoflurane and thiopental were from Cristália (Itapira, SP, Brazil).

Venoms and Antivenom. The venoms of *V. dubius* (Rocha-e-Silva et al., 2009b), *Phoneutria nigriventer* (wandering spider), and *Tityus serrulatus* (yellow scorpion) were obtained by electrical stimulation of specimens of both sexes. Antiarachnid antivenom produced by the Instituto Butantan (São Paulo, SP, Brazil) and obtained by immunizing horses with a pool of spider (*P. nigriventer* and *Loxosceles gaucho*) and scorpion (*Tityus serrulatus*) venoms (Cardoso et al., 2003) was provided by the Poison Control Center of the university teaching hospital at Universidade Estadual de Campinas.

Rat Dorsal Skin Edema. Rats were anesthetized with thiopental (60 mg/kg, i.p.) and maintained anesthetized throughout the experiments, with additional anesthetic being given when required. After the induction of anesthesia, the dorsal skin was shaved, and 1, 3, 10, 30, and 100 μg venom was injected intradermally (100 μl/site in Tyrode solution). The injections were done in a random order using a balanced site pattern. Plasma protein extravasation was measured by the accumulation of i.v. injected ^{125}I-human serum albumin (2.5μCi/rat) with Evan's blue dye (25mg/kg; Brain and Williams, 1985) to act as a visual marker. Thirty minutes after venom injection, a blood sample (1 ml) was obtained immediately before the rats were killed with an overdose of anesthetic and the sample then centrifuged (8000*g*, 10 minutes) to obtain plasma. The injected sites were subsequently punched out and counted for radioactivity, along with the plasma sample, in a gamma counter (Antunes et al., 1993; Marangoni et al., 1993). Plasma extravasation was expressed as the volume (μl) of plasma accumulated at each skin site compared with the total counts in 1ml plasma.

To evaluate the mechanisms involved in the edema induced by venom (10 μg/site), different drugs were administered, as follows: cyproheptadine (dual serotonin 5-hydroxytryptamine$_{1/2}$ and histamine H_1 receptor antagonist; 2 mg/kg, i.p., 30 minutes before; Câmara et al., 2003), JE 049 (bradykinin B_2 receptor antagonist; 0.6 mg/kg, i.v., 15 minutes before; Costa et al., 2001), indomethacin (nonselective COX inhibitor; 10 mg/kg, i.p., 1 hour before; Barbosa et al., 2003), L-NAME (nitric oxide synthase inhibitor; 100 nmol/site, coinjected; Ridger et al., 1997), mepyramine (histamine H_1 receptor antagonist; 6 mg/kg, i.p., 15 minutes before; Barbosa et al., 2003), SR140333 (neurokinin NK_1 receptor antagonist; 1 nmol/site, coinjected; Câmara et al., 2003), and SR48968 (neurokinin NK_2 receptor antagonist; 0.3 μmol/kg, i.v.).

The ability of antiarachnid antivenom to neutralize the edema-forming activity of *P. nigriventer*, *T. serrulatus*, and *V. dubius* venoms was assessed by incubating venom samples with IgG purified from antiarachnidic antivenom by liquid chromatography using a protein G affinity column. After purification, the IgG fraction was lyophilized to allow weighting. The amount of *P. nigriventer* and *T. serrulatus* venoms to be injected per site was determined from preliminary experiments using various amounts of venom and found to be 5 μg/site because this produced plasma extravasation similar to that obtained with 10 μg *V. dubius* venom/site. The IgG concentrations used in the neutralization assays were 0.3 mg/ml for *V. dubius* venom (1:3, venom:IgG) and 0.1 mg/ml for *P. nigriventer* and *T. serrulatus* venoms (1:2, venom:IgG), based on the differential reactivity described by Rocha-e-Silva et al. (2009b). The mixtures of Igs and venom were preincubated for 1 hour at 37°C before injecting into the dorsal skin.

Rat Hind Paw Edema. Rats were anesthetized with isoflurane, and venom (20 μg/paw) was injected in the subplantar region of the right hind paw. The paw volume was measured immediately before venom injection and 30, 60, 90, 120, and 180 minutes postvenom using a plethysmometer (model 7150; Ugo Basile, Varese, Italy). The volume injected into the paws was always 100 μl. The control group received the same volume of vehicle solution (0.9% NaCl). The increase in paw volume (ml) was calculated as the difference between the basal and final volumes. To evaluate the effect of ketoprofen, a nonselective COX inhibitor, on venom-induced edema, the drug (20 mg/kg, i.p.) was administered 15 minutes after venom or saline (vehicle) injection into the paw. The control group received saline without ketoprofen.

Statistical Analysis. The results are expressed as the mean ± S.E.M. One-way analysis of variance was used for dorsal skin edema and two-way analysis of variance for hind paw edema, followed by the Tukey test in both cases. A value of $P < 0.05$ indicated significance.

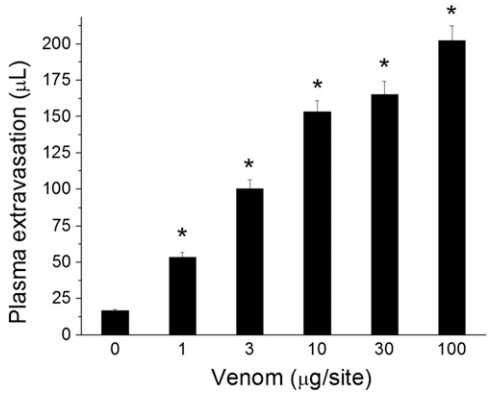

Fig. 1. Rat dorsal skin plasma extravasation caused by *V. dubius* venom. The rats were anesthetized with thiopental, the dorsal skin was shaved, and the animals received an i.v. injection of ^{125}I-human serum albumin (2.5μCi/rat) with Evan's blue dye (25mg/kg). This was followed by the intradermal injection of vehicle (Tyrode) solution or venom (1–100 μg/site) in a fixed volume (100μl) using a random order and balanced site pattern. Thirty minutes later, the rats were euthanized, the dorsal skin was removed, and the injected sites were punched out and counted in a gamma counter to determine the extent of plasma extravasation (based on the accumulation of radiolabeled albumin). Plasma extravasation was expressed as the volume (μl) of plasma accumulated at each skin site. The columns represent the mean ± S.E.M. (n = 6). *$P < 0.05$ compared with vehicle (Tyrode) solution (column with 0 μg venom/site).

Results

Figure 1 shows that *V. dubius* venom caused dose-dependent edema in rat dorsal skin, with the plasma extravasation increasing by nearly 11-fold with the highest dose (from 17 ± 1 μl for the Tyrode control to 202 ± 10 μl for 100 μg/site); even at this highest dose there was no apparent plateau in the curve. Based on this dose-response curve, a venom dose that produced an intermediate edematogenic response (10 μg/site) was chosen for subsequent experiments. Postmortem examination of the rats used in this protocol revealed no macroscopic alterations or damage to internal organs, including the heart, lungs, kidney, and liver; no microscopic (histologic) analysis of these organs was undertaken.

The potential mediators of the venom-induced edema in rat dorsal skin were investigated using antagonists and doses reported in similar studies in the literature. Mepyramine (a histamine H_1 receptor antagonist) and JE 049 (a bradykinin B_2 receptor antagonist) had no effect on the venom-induced edema. In contrast, cyproheptadine (a dual histamine and serotonin receptor antagonist) abolished the plasma extravasation to the level seen with saline alone (plasma extravasation from 76 ± 6 μl with venom to 36 ± 8 μl with venom + cyproheptadine; n = 6 each), and indomethacin partially inhibited the edema from 108 ± 10 μl with venom alone to 61 ± 15 μl with venom plus indomethacin (n = 6 each) (Fig. 2). Treatment with L-NAME (a nitric oxide synthase inhibitor) reduced the edema by ~50% (Fig. 3). These results indicate that histamine and bradykinin are not involved in venom-induced edema, but that serotonin, nitric oxide, and COX products are probably involved.

The involvement of neurokinin receptors was investigated using SR140333 and SR48968, neurokinin NK_1 and NK_2 receptor antagonists, respectively. Treatment with the former antagonist resulted in a ~43% reduction in edema, whereas the latter antagonist had no effect on the edema (Fig. 3). This finding indicates a role for tachykinins in venom-induced edema and suggests that this effect is mediated specifically by NK_1 neurokinin receptors.

To increase our understanding of the involvement of COX products in venom-induced edema, we examined the ability of *V. dubius* venom to cause edema in rat hind paw. This model has an advantage over the dorsal skin model in that it allows time-course curves to be obtained in the same animals. In rat hind paw, *V. dubius* venom (20 μg/paw) caused marked edema that was maximal at 30 minutes and decreased progressively thereafter over the ensuing 150 minutes (Fig. 4). Treatment with ketoprofen given 15 minutes postvenom, that is, before the first measurement of edema postvenom, did not significantly affect the peak edematogenic responses (30 minutes postvenom) but significantly hastened the recovery from venom-induced edema at the later postvenom intervals. At 3 hours (180 minutes) postvenom, edema formation in the venom plus ketoprofen group was significantly lower than with venom alone (0.15 ± 0.03 ml and 0.30 ± 0.05 ml, respectively; n = 6 each).

P. nigriventer and *T. serrulatus* venoms (5 μg/site each) produced plasma extravasation that was not significantly different from that caused by *V. dubius* (10 μg/site), despite the slightly smaller response with *P. nigriventer* venom. Preincubation with purified antiarachnid IgG in a venom:IgG ratio of 1:2 abolished the edema caused by *P. nigriventer* and *T. serrulatus* venoms (positive controls), whereas a venom:IgG ratio of 1:3 only partially and nonsignificantly attenuated the plasma extravasation produced by *V. dubius* venom (Fig. 5).

Discussion

Although bites by theraphosids can produce local effects such as pain, erythema, and edema (Lucas et al., 1994), few studies have examined the mediators and mechanisms involved in the edema caused by these venoms. As shown in this work, *V. dubius* venom caused edema in rat dorsal skin and hind paw, with the latter being similar to the rat paw edema reported for venom of the theraphosid *Acanthoscurria paulensis*; in this species, venom (20–60 μg/paw) produced

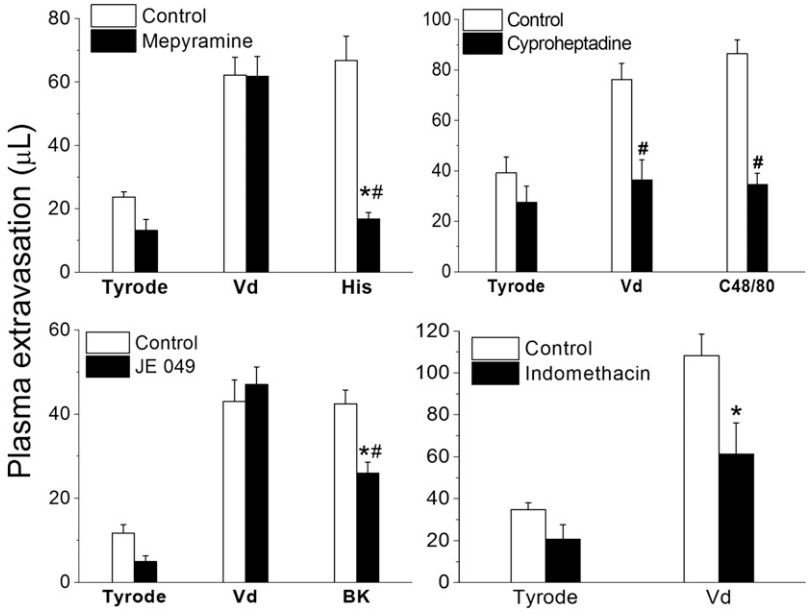

Fig. 2. The role of histamine, serotonin, bradykinin, and COX products in *V. dubius* (Vd) venom-induced plasma extravasation. The rats were prepared and treated as described in the legend for Fig. 1, except that prior to venom injection they received mepyramine (histamine H_1 receptor antagonist; 6 mg/kg, i.p., 15 minutes before), cyproheptadine (dual serotonin 5-hydroxytryptamine$_{1/2}$ and histamine H_1 receptor antagonist; 2 mg/kg, i.p., 30 minutes before), JE 049 (B_2 receptor antagonist; 0.6 mg/kg, i.v., 15 minutes before), or indomethacin (nonselective COX inhibitor; 10 mg/kg, i.p., 1 hour before). Subsequently, vehicle (Tyrode) solution, the respective positive control, and venom (10 μg/site) were injected intradermally in a fixed volume (100μl). Thirty minutes later, the rats were euthanized and processed, as described in Fig. 1. Plasma extravasation was expressed as the volume (μl) of plasma accumulated at each skin site. Histamine (His, 30 nmol/site), compound 48/80 (C48/80, 1 μg/site), and bradykinin (BK, 0,1 nmol/site?) were used as positive controls. The columns represent the mean ± S.E.M. (n = 6). $^{*}P < 0.05$ compared with vehicle (Tyrode) solution and $^{\#}P < 0.05$ compared with the corresponding response to venom alone.

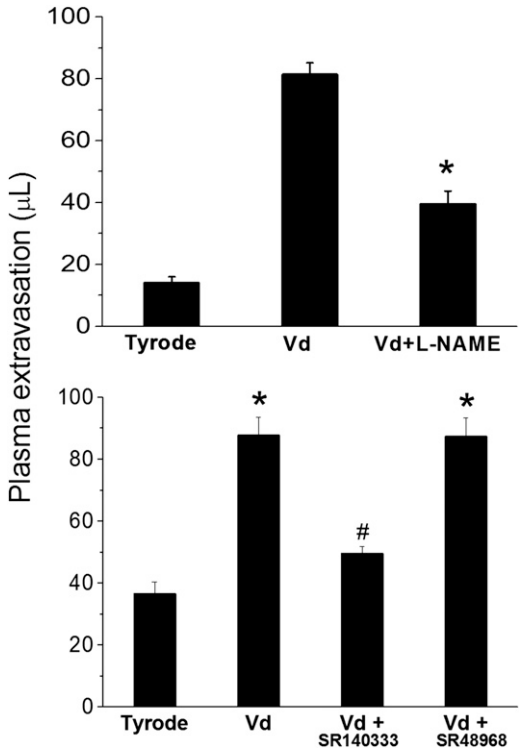

Fig. 3. Role of nitric oxide and neurokinins in *V. dubius* (Vd) venom-induced plasma extravasation. The rats were prepared and treated as described in the legend for Fig. 1, except that prior to venom injection they received (Upper panel) L-NAME (nitric oxide synthase inhibitor; 100 nmol/site) and (Lower panel) SR140333 (neurokinin NK_1 receptor antagonist; 1 nmol/site) or SR48968 (neurokinin NK_2 receptor antagonist; 0.3 μmol/kg, i.v.) that were coadministered with venom (10 μg/site) in a fixed volume (100μl). Thirty minutes later, the rats were euthanized and processed as described in Fig. 1. Plasma extravasation was expressed as the volume (μl) of plasma accumulated at each skin site. The columns represent the mean ± S.E.M. (n = 6). $^*P < 0.05$ compared with vehicle (Tyrode) solution and $^{\#}P < 0.05$ compared with the corresponding response to venom alone.

dose-dependent edema that was significant within 10 minutes postvenom and lasted for at least 2 hours (Mourão et al., 2013). Edema formation has also been observed for nontheraphosid spiders such as *P. nigriventer* (Antunes et al., 1992, 1993; Bento et al., 1995; Costa et al., 1997) and *Loxosceles* spp. (Rattmann et al., 2008; Paludo et al., 2009; Barbaro et al., 2010; Guimarães et al., 2013; Ribeiro et al., 2015), and a variety of Australian spiders (Atkinson, 1986; Korszniak and Story, 1995). Unlike envenoming by *Loxosceles* spp. (Futrell, 1992; Tambourgi et al., 2010), *V. dubius* venom caused no macroscopic local dermonecrosis; there were also no macroscopic alterations or damage to internal organs. This lack of local and systemic lesions agrees with the absence of proteolytic (caseinolytic, elastolytic, and collagenolytic) activity in this venom (Rocha-e-Silva et al., 2009b).

To examine the mediators involved in the venom-induced edema, rats were treated with various antagonists before or concomitantly with venom. Histamine contained in the venom or released by mast cell degranulation by venom components could potentially contribute to venom-induced edema. However, as shown in this work, treatment with mepyramine, a histamine H_1 receptor antagonist, had no effect on *V. dubius* venom-induced edema, indicating no role for this mediator in this response. Although there has been no systematic screening for the presence of histamine in theraphosid venoms, mass spectrometric analysis of venom from *Hapopelma lividum* indicated only a very low content of histamine (0.14%) in this venom (Moore et al., 2009). In contrast, histamine is involved in *P. nigriventer* venom-induced edema in rat skin (Palframan et al., 1996) and hind paw (Costa et al., 2000, 2001) following the activation of mast cells. Histamine has a role in the vascular permeability caused by *Atrax* spp. venoms (Atkinson, 1986) but is not a major mediator in edema caused by other Australian spider venoms (Korszniak and Story, 1995).

In contrast to the lack of involvement of histamine, serotonin was an important mediator of *V. dubius* venom-induced edema because cyproheptadine (a mixed serotonin/histamine receptor antagonist) significantly attenuated the venom-induced edema. Atkinson (1986) reported that the edema caused by *Atrax* sp. venoms in rats resulted from the degranulation of mast cells in the microcirculation, leading to the release of histamine and serotonin. The use of a mixture of histamine and serotonin antagonists abolished the edema, whereas lesions to the sensory nerve did not affect the edema. Costa et al. (2000) have also noted that the blockade of histamine and serotonin receptors attenuated the edema caused by *P. nigriventer* venom.

Tachykinins (substance P and neurokinins A and B) exert a variety of functions in the peripheral nervous system, including the modulation of vascular permeability and pain. Substance P acts though NK_1 receptors, whereas neurokinins A and B act through NK_2 receptors. Tachykinins have been implicated in the edema caused by venoms of the spider *P. nigriventer* (Palframan et al., 1996; Costa et al., 1997, 2001) and the social wasp *Polistes lanio lanio* (Yshii et al., 2009), and in itching caused by *P. nigriventer* venom (Costa et al., 2006). Because these responses are abolished by blockade of NK_1 but not NK_2 receptors, and attenuated in NK_1 knockout mice, the conclusion is that substance P is the main mediator involved. The activation of NK_1 receptors by *P. nigriventer* venom is apparently indirect, following the stimulation of sensory nerves to release substance P, rather than through direct stimulation by tachykinin-like peptides in the venom (Costa et al., 1997). Although tachykinin-like peptides have been identified in the venoms of *P. nigriventer* (Pimenta et al., 2005) and *P. l. lanio* (Yshii et al., 2009), the extent to which they contribute to venom-induced edema is unclear.

Our findings for *V. dubius* agree with this general pattern regarding a role for NK_1 but not NK_2 receptors in arthropod venom-induced edema. However, our experimental protocol did not allow us to distinguish between direct activation of the receptors by venom peptides or indirect activation through the release of substance P after sensorial nerve stimulation. In contrast to venom-induced edema, the hyperalgesia caused by *P. nigriventer* venom involves both peripheral and central NK_1 and NK_2 receptors (Zanchet and Cury, 2003; Zanchet et al., 2004), whereas the venoms of the Trinidad Chevron tarantula (*Psalmopoeus cambridgei*) (Siemens et al., 2006) and the Chinese bird spider (*Ornithoctonus huwena*) (Bohlen et al., 2010) contain toxins (vanillotoxins—VaTx1, 2, and 3, and a "double-knot" toxin—DkTx, respectively) that activate capsaicin-sensitive TRPV1 receptors. A role for vanilloid receptor activation in modulating the edema induced by *P. nigriventer* venom has also been suggested (Costa et al., 2000).

In contrast to the involvement of tachykinin NK_1 receptors, the lack of effect of JE 049 (Icatibant, HOE-140), a bradykinin

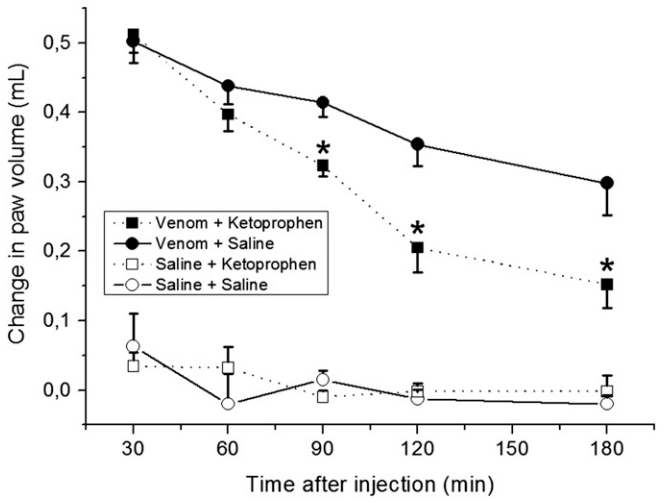

Fig. 4. Attenuation of *V. dubius* venom-induced rat hind paw edema by ketoprofen (cyclooxygenase inhibitor; 20 mg/kg, i.p.) administered 15 minutes after venom. Rats were lightly anesthetized with isoflurane for venom injection in the subplantar region of the right hind paw. The paw volume was measured immediately before the injection of venom (20 μg/paw) or 0.9% NaCl (vehicle control) and at various intervals thereafter using a plethysmometer. The volume injected into the paws was always 100 μl. The increase in paw volume (ml) was calculated as the difference between the basal and final volumes. The points represent the mean ± S.E.M. (n = 6). All time points in the venom + saline and venom + ketoprofen groups were significantly different from the corresponding controls. *$P <$ 0.05 compared with the corresponding times in the venom + saline group.

B_2 receptor antagonist, on the venom-induced edema indicated that kinins were not involved in the edematogenic response to *V. dubius* venom. Kinins are apparently also not involved in the edema induced by *P. nigriventer* venom (Costa et al., 2000, 2001). However, in other spider venoms, for example, *Scaptocosa raptorial* (Ferreira et al., 1998), kinin-like peptides may be important mediators of venom-induced edema. Kinins are also not involved in the mouse dorsal skin edema caused by *P. l. lanio* wasp venom (Yshii et al., 2009).

The ability of L-NAME, a nonselective inhibitor of nitric oxide synthase, to attenuate the venom-induced edema indicated a role for nitric oxide in this response. Nitric oxide production could be stimulated directly by *V. dubius* venom components, for example, peptides, or indirectly via mediators released by the venom. Endothelium-derived nitric oxide has also been implicated in the vasodilation caused by *Lasiodora* sp. venom (Horta et al., 2013).

In addition to the mediators (serotonin, nitric oxide, COX products, and tachykinins/substance P) shown to be involved in *V. dubius* venom-induced edema, other mediators not examined in this work may also have a role. For example, adenosine 5′-diphosphate is the principal mediator of edema caused by venom of the theraphosid *Lasiodora* sp. (Horta et al., 2013), whereas adenosine 5′-diphosphate and ATP may actually inhibit edema caused by *P. nigriventer* venom (Costa et al., 2000).

The finding that the nonselective COX inhibitors indomethacin and ketoprofen significantly attenuated the venom-induced edema in rat dorsal skin and hind paw, respectively, indicated an important role for COX products, primarily the vasodilators prostacyclin and prostaglandin E_1, in this response. The finding for ketoprofen is of practical relevance as it suggests that COX inhibitors may be useful for treating venom-induced edema after theraphosid bites. Whereas the observation that the hind paw edema was significantly attenuated only in its later stages of development may indicate that this is the phase in which COX products are acting, it may also simply reflect the kinetics of ketoprofen absorption and the time required for COX inhibition rather than being an indicator of a lack of involvement of COX products in the early phase of the edema. The ability of COX inhibitors to attenuate arthropod venom-induced edema has also been reported for experimental envenoming with *P. nigriventer* (Zanchet et al., 2004) and *T. serrulatus* (Nascimento Jr. et al., 2005) venoms.

Antivenom is standard treatment of envenoming by spiders (e.g., *P. nigriventer* and *Loxosceles* spp.) (Bucaretchi et al., 2000, 2008; Bucaretchi and Hyslop, 2009) and scorpions (*Tityus* spp.) (Bucaretchi et al., 2014) in Brazil. The finding that the increase in vascular permeability caused by *P. nigriventer* and *T. serrulatus* venoms was abolished by antivenom agreed with the demonstration by Toro et al. (2006) that IgG partially neutralized the edematogenic activity of these two venoms and indicated that the venom components responsible for this activity were peptides/polypeptides that were sufficiently immunogenic so as to stimulate antibody production in horses. We have previously shown, using ELISA and immunoblotting, that *V. dubius* venom also cross-reacts with this antiarachnid antivenom produced by the Instituto Butantan (Rocha-e-Silva et al., 2009b). Immunoblotting showed that only venom components >10 kDa reacted with the antivenom (Rocha-e-Silva et al., 2009b), including hyaluronidase (45 kDa) (Sutti et al., 2014). Studies with *T. serrulatus* scorpion venom have shown that hyaluronidase has an important role in the action of this venom (Pessini et al., 2001; Horta et al., 2014), and we have also observed the *V. dubius* enzyme to be edematogenic. However, in contrast to *P. nigriventer* and *T. serrulatus* venom-induced edema, the response to *V. dubius* venom was only partially neutralized by antivenom, perhaps indicating incomplete immunologic identity between the theraphosid venom components responsible for

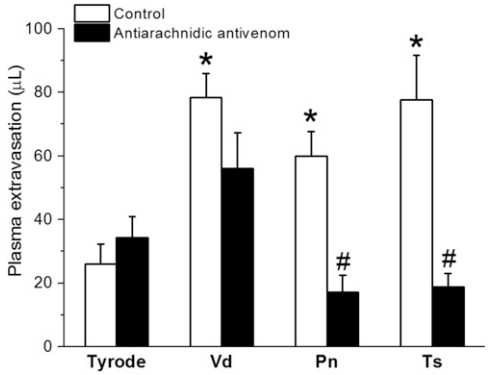

Fig. 5. Neutralization of *V. dubius* (Vd, 10 μg/site), *P. nigriventer* (Pn, 5 μg/site), and *T. serrulatus* (Ts, 5 μg/site) venom-induced plasma extravasation by IgG purified from antiarachnid antivenom. The venoms were incubated with IgG purified from antiarachnid antivenom (0.3 mg/ml for *V. dubius* and 0.1 mg/ml for *P. nigriventer* and *T. serrulatus*) for 1 hour at 37°C prior to testing in rat dorsal skin. The venom:IgG ratios used for these neutralization assays were 1:2 for *P. nigriventer* and *T. serrulatus* and 1:3 for *V. dubius*. Plasma extravasation was assessed by injecting venom and venom + antivenom IgG mixtures intradermally in the dorsal skin of anesthetized rats. Thirty minutes later, the rats were euthanized and processed, as described in Fig. 1. Plasma extravasation was expressed as the volume (μl) of plasma accumulated at each skin site. The columns represent the mean ± S.E.M. (n = 6). *$P <$ 0.05 compared with vehicle (Tyrode) solution and $^{\#}P <$ 0.05 compared with the corresponding response to venom alone.

causing edema and those of *P. nigriventer* and *T. serrulatus* venoms used to produce the antivenom, or that *V. dubius* venom contains peptides/proteins that are not present in the venoms used for antivenom production. Although antiarachnid antivenom partially neutralized the edema-forming activity of *V. dubius* venom, the mild clinical symptoms associated with bites by most Brazilian theraphosids means that antivenom therapy is unlikely to be used, with treatment being essentially symptomatic. In addition, the risk of the patient developing a hypersensitivity reaction or serum sickness in response to antivenom considerably outweighs the potential clinical benefits of this treatment. In this case, the administration of a nonsteroidal anti-inflammatory drug such as ketoprofen could be a useful alternative.

In conclusion, we have shown that *V. dubius* venom causes local edema in two rat models, and this edema is dependent on COX products, nitric oxide, serotonin, and tachykinins, but not on histamine or kinins. The attenuation of hind paw edema by ketoprofen suggests that COX inhibitors may be useful for treating the local responses to envenoming by theraphosids in animals and humans.

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Authorship Contributions

Participated in research design: Rocha-e-Silva, Linardi, Hyslop.

Conducted experiments: Rocha-e-Silva, Linardi.

Contributed new reagents or analytic tools: Hyslop, Antunes.

Wrote or contributed to the writing of the manuscript: Rocha-e-Silva, Linardi, Antunes, Hyslop.

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