Ctenus medius and *Phoneutria nigriventer* Spiders Venoms Share Noxious Proinflammatory Activities

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ABSTRACT *Ctenus medius* Keyserling, 1891 (Araneae: Ctenidae) co-occurs in various microhabitats of the Brazilian Atlantic Forest and can be easily misidentified as the medically important spider *Phoneutria nigriventer* Keyserling, 1981 (Ctenidae). Despite being phylogenetically close to *Phoneutria*, no data are available about the toxic potential of *Ctenus medius* venom. Here we show that, although presenting different profile of protein composition, *C. medius* venom displays some of the toxic properties exhibited by *P. nigriventer* venom, including proteolytic, hyaluronidasic and phospholipasic activities, as well as the ability of causing hyperalgesia and edema. Moreover, *C. medius* venom interferes in the activation of the complement system in concentrations that *P. nigriventer* venom is inactive. Thus, these data show that venoms of spiders from Ctenidae family share important proinflammatory properties and suggest that the *C. medius* bite may have an important noxious effect in human accidents.

KEY WORDS *Ctenus medius*, *Phoneutria nigriventer*, spider venoms, proinflammatory activities, complement system

Spider venoms are predominantly mixtures of protein and peptide toxins. In addition, polyamine neurotoxins, free amino acids, monoamines, and inorganic salts are also present (Jackson and Parks 1989, Rash and Hodgson 2002). Spiders of the genera *Trechona* (Dipluridae), *Atrax* (Hexathelidae), *Harpactirella* (Theraphosidae), *Loxosceles* (Sicariidae), *Latrodectus* (Theridiidae), and *Phoneutria* (Ctenidae) have been reported to cause lethal accidents (Habermehl and Mebs 1984).

The Brazilian "armed" spider *Phoneutria nigriventer* Keyserling, 1891 is extremely aggressive, and its bite is reported to cause severe and irradiating pain and several toxic symptoms, such as cramps, convulsions, spastic paralysis, priapism, sialorrhea, arrhythmia, visual disturbance, and cold sudoresis (Brazil and Vellard 1925, Schenberg and Lima 1966, Gomez et al. 2002).

Ctenus medius Keyserling, 1891 is a medium-to-large-sized ctenid spider (Almeida et al. 2000). This wandering spider is endemic in the Brazilian Atlantic rainforest (Brescovit and Simó 2007). It is a sit-and-wait predator with nocturnal habits, and it is mainly found on the forest litter. During the day, it hides under fallen trunks, among leaves in the litter, under bark, and other retreats that aid it in protecting against light. Although the interaction between humans and this spider has not been reported, *C. medius* co-occurs with the medical important spider, *Phoneutria* spp., in the various microhabitats that compose the Brazilian Atlantic Forest. In undisturbed areas, its population outnumbers that of *Phoneutria* spp. The spider *C. medius* has a sexual dimorphic color pattern, and in the spiderlings, this pattern changes depending on their developmental stadium. Human envenomation caused by *C. medius* bite has not been reported, although this species can be easily misidentified as *Phoneutria* spp., especially the male, because of its large size and color pattern (Almeida et al. 2000).

Ctenus medius is 1 of the 250 described species in the genus (Platnick 2008), which comprises more than one half of the total species in the Ctenidae. The huge number of species in *Ctenus* is because of the lack of taxonomic knowledge both on the genus and in the family. *Ctenus* needs urgent taxonomic revision, and it is expected that its species will be split into several genera in the future, because the genus is clearly polyphyletic (Silva 2003). Because of the absence of well-elaborated and profound studies on *Ctenus* taxonomy, it is common for species of the genus to be placed into different ctenid genera and even in other families when new evidence is found that indicates one particular species is more or less related to one or

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another genus. This caused confusion in the medical literature in the past, when the genus *Phoneutria* was synonymized with *Ctenus* by Walckenaer (1837) and revalidated almost a century thereafter by Mello-Leitão (1936). Something similar happened with *C. medius.* The species was firstly transferred to *Oligoctenus* Simon by Lehtinen (1967), and recently the genus was synonymized with *Ctenus* by Brescovit and Simó (2007), returning to its original genus. The occurrence of these taxonomic transferences should be kept in mind by medical and toxinological researchers when searching and comparing papers published over a long period.

Phoneutria spp. is the second most important cause of spider bites in Brazil. The incidence of the accidents is higher in March and April and is probably related to the mating period of these spiders, when they can be more easily seen and captured (Ramos et al. 1998). Males of *C. medius* are also easily seen in March and April, which indicates that both species have a similar reproductive season. Their similarity, parallel geographic distribution, and concomitant reproductive season can lead to mistakes in their identification in cases of bites.

Despite being phylogenetically close to *Phoneutria* (Simó and Brescovit 2001, Silva 2003) and abundant in the Brazilian Atlantic Forest, no data are available on the toxic potential of the venom of *C. medius.* Therefore, the aim of this study was to compare the toxic potential of venoms from *C. medius* and *P. nigriventer* spiders.

Materials and Methods

Chemicals and Reagents. Tween 20, bovine serum albumin (BSA), Triton X-100, phenylmethylsulfonyl fluoride (PMSF), phenanthroline, 4-nitro-3(octanoyloxy) benzoic acid, and hyaluronic acid were purchased from Sigma (St. Louis, MO). Goat anti-horse (GAH) IgG labeled with alkaline phosphatase (IgG-AP), 5-bromo-4-chloro-3-indolyl-phosphate (BCIP), and nitroblue tetrazolium (NBT) were purchased from Promega (Madison, WI). Fluorescent resonance energy transfer (FRET) substrate, Abz-FRSSRQ-EDDnp, was synthesized and purified according to Hirata et al. (1994).

Venoms. *Ctenus medius* spiders were collected in forest areas of the Parque Estadual Turístico do Alto Ribeira, Iporanga, SP, Brazil (capture licenses numbers: 40320/2002 from Florestal Institute, SP, Brazil, and 20/2002 from IBAMA, Brazil). *P. nigriventer* and *Loxosceles gaucho* Gertsch, 1967 are being currently kept and bred in the scientific reproduction room of the Immunochemistry Laboratory, Butantan Institute, SP, Brazil. Venoms were obtained by electrostimulation from female spiders by the method of Bucherl (1969), with slight modifications. Briefly, 15- to 20-V electrical stimuli were repeatedly applied to the spider sternum, and the venom drops were collected with a micropipette, vacuum dried, and stored at −20°C. Only female venom was used, because intersexual variations in the toxic potential of *P. nigriventer* have already been shown; female venom is more poisonous than male venom (Herzig et al. 2002). The protein content of the samples was evaluated by the method of Smith et al. (1985), using the BCA Protein Assay kit (Pierce Biotechnology, Rockford, IL). Stock solutions were prepared in phosphate-buffered saline (PBS; 10 mM sodium phosphate, 150 mM NaCl, pH 7.2) at 1.0 mg/ml. Venom from *Crotalus durissus terrificus*, which was used as positive control in the assays for determination of PLA_2 activity, was supplied by Herpetology Laboratory (Butantan Institute, SP, Brazil).

Animals. Male Wistar rats, weighing 160–180 g, were obtained from the Central Animal Breeding Laboratory from Butantan Institute, SP, Brazil. All the procedures involving animals were in accordance with the ethical principles in animal research adopted by the Brazilian College of Animal Experimentation.

Normal and Hyperimmune Sera. The anti-arachnidic serum, produced by hyperimmunization of horses with venoms from the scorpion *Tityus serrulatus* Lutz and Mello, 1922 (57%) and the spiders *P. nigriventer* (21.5%) and *L. gaucho* (21.5%), was obtained from Seção de Processamento de Plasmas Hiperimunes, Butantan Institute, SP, Brazil. Normal sera were obtained from nonimmunized animals.

Electrophoresis and Western Blot. Samples of 20 μg of *C. medius* and *P. nigriventer* venoms were solubilized in nonreducing sample buffer and run on 12% SDS-PAGE (Laemmli 1970). Gels were stained with silver (Morrissey 1980) or blotted onto nitrocellulose (Towbin et al. 1979). After transfer, the membranes were blocked with PBS containing 5% BSA and incubated with anti-arachnidic or normal horse serum (1:2,000) for 1 h at room temperature. Membranes were washed three times for 10 min with PBS/0.05% Tween 20 and incubated with GAH/IgG-AP (1:7,500) in PBS/1% BSA for 1 h at room temperature. After washing three times for 10 min with PBS/0.05% Tween 20, blots were developed using NBT/BCIP, according to the manufacturer's instructions (Promega).

Phospholipase A_2 Activity. The PLA_2 activity of *C. medius* and *P. nigriventer* venoms was measured according to Beghini et al. (2000), with slight modifications. Samples of venoms (10 μg) were mixed with 100 μl of buffer (10 mM Tris-HCl, 10 mM $CaCl_2$, 100 mM NaCl, pH 8.0), 0.3 mM (25 μl) of the substrate 4-nitro-3 (octanoyloxy) benzoic acid, and milli-Q H_2O for a final volume of 150 μl. The mixtures were incubated at 37°C for up to 60 min, with the absorbance (λ414 nm) being determined at 10-min intervals, in a spectrophotometer (Multiskan-EX; Labsystems, Helsinki, Finland). Venom from *Crotalus durissus terrificus* (Laurenti, 1768) (10 μg) and PBS were used as positive and negative controls, respectively. The specific PLA_2 activity was expressed as nanomoles per minute per milligram of venom.

Proteolytic Activity. The enzymatic activity of the *P. nigriventer* and *C. medius* venoms was determined in a continuous assay using FRET peptide as substrate in the presence or absence of inhibitors. Samples of the venoms (0.1 or 1 μg) were mixed with 2 μM of the quenched fluorescent peptide Abz-FRSSRQ-EDDnp,

and the relative inhibition was determined in parallel with 5 mM PMSF or 5 mM phenanthroline, inhibitors of serine proteases and metalloproteases, respectively. The stock solutions and the work concentration of the synthetic inhibitors used in the characterization of the venoms proteolytic activity were made as described (Beynon and Bond 2001). The reactions were monitored by measuring (fluorescence at $\lambda_{em} = 420$ nm and $\lambda_{ex} = 320$ nm) the hydrolysis in a fluorescence spectrophotometer (Victor 3; Perkin-Elmer, Boston, MA) at 37°C, as described by Araújo et al. (2000). Control samples were made in the presence of the same volume of methanol used in inhibitors stock solutions. The specific proteolytic activity was expressed as units of free fluorescence per minute of cleaved substrate per microgram of venom.

Alternatively, venoms samples (0.1 or 1 μg) were incubated with the purified component C3 (9 μg) of the complement system in the presence or absence of 5 mM of the protease inhibitors, i.e., PMSF or phenathroline, for 2 h at 37°C. C3 cleavage was visualized in SDS-PAGE gels, developed under reduction conditions, and stained with Coomassie blue.

Hyaluronidase Activity. Hyaluronidase activity was measured following the procedures of Pukrittayakamee et al. (1988) with slight modifications. Samples of *C. medius* and *P. nigriventer* venoms (10 μg) were added to 100 μl of the hyaluronic acid substrate (1 mg/ml) and acetate buffer (pH 6.0) for a final volume of 500 μl. The mixtures were incubated for 15 min at 37°C. After incubation, 1 ml cetyltrimethylammonium bromide (Sigma) 2.5% in NaOH 2% was added to the samples to develop the turbidity in the mixtures, and the absorbance was measured in a spectrophotometer (Multiskan-EX; Labsystems) at $\lambda_{em} = 405$ nm. Venom from the spider *L. gaucho* (10 μg) was used as a positive control.

Normal Human Serum and Sheep Erythrocytes. Human blood was obtained from healthy donors. Blood samples drawn to obtain sera were collected without anticoagulant and allowed to clot for 2 h at room temperature; the normal human serum (NHS) was stored at −80°C. Blood samples from sheep, drawn to obtain erythrocytes (E) for subsequent use as target cells in complement assays, were collected in anticoagulant (Alsever's old solution: 114 mM citrate, 27 mM glucose, 72 mM NaCl, pH 6.1).

Hemolytic Complement Assay. Samples of 50 μl NHS, as source of complement components, were incubated with 50 μl of the spider venom (500 μg/ml) or with PBS (control samples) for 1 h at 37°C and assayed for the residual complement activity, using a hemolytic test. For this, sheep erythrocytes (E^s) were washed in PBS, and a 2% suspension was incubated with an equal volume of a 1:500 dilution, in PBS, of rabbit anti-sheep erythrocyte antiserum for 15 min at 37°C. The antibody-sensitized sheep erythrocytes were washed and resuspended in veronal buffered saline (VBS^{++}: 10 mM Na barbitone, 0.15 mM $CaCl_2$, and 0.5 mM $MgCl_2$, pH 7.4) at a 2% final concentration. For each serum to be tested, doubling dilutions in VBS^{++} were made in the wells of a 96-well plate (150 μl/well), including 0% and 100% lysis wells. The antibody-sensitized sheep erythrocytes (50 μl) were added to each well, and the plate was incubated for 30 min at 37°C. Fifty microliters of each supernatant was transferred to new 96-well plates containing 200 μl water, and the absorbance was measured at 414 nm as an index of hemolysis. Percentage of hemolysis for each sample was calculated by standard methods.

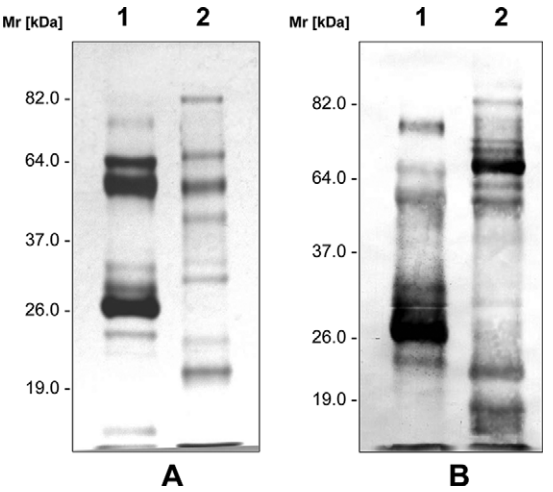

Fig. 1. SDS-PAGE and Western blot analysis of *C. medius* and *P. nigriventer* venoms. (A) Samples of *C. medius* (1) and *P. nigriventer* (2) venoms (20 μg) were submited to electrophoresis on a 12% SDS-PAGE gel under nonreducing conditions and silver stained or Western blotted. (B) Blots were probed with anti-arachnidic horse serum (diluted 1:2,000) followed by GAH/IgG-AP, and the reaction was developed using NBT/BCIP.

Evaluation of Mechanical Hyperalgesia (Randall-Selitto Test). Hyperalgesia was induced by intraplantar (i.pl.) administration of 100 μl of sterile saline (0.85% NaCl solution) alone or containing venoms from *C. medius* (0.25, 0.1, 0.5, or 1.0 μg) or *P. nigriventer* (1.0 μg) into one of the rat hind paws. Pain threshold was measured before and at different times after injections, using an Ugo-Basile pressure apparatus (Randall and Selitto 1957). Tests were blind, in regard to group designation, and were performed by increasing the force (*g*) applied to the rat hind paw. The force needed to induce paw withdrawal was recorded as the pain threshold. To reduce stress, rats were habituated to the testing procedure the day before the experiment.

Evaluation of Edema. Edema was induced by i.pl. administration of 0.1 ml of sterile saline (0.85% NaCl solution) alone or containing venoms from *C. medius* (1.0 μg) or *P. nigriventer* (1.0 μg) into one of the hind paws. Saline (0.1 ml, control) was injected into the contralateral paw. The increase in paw volume (edema) was determined by measuring paw thickness using a caliper at 0 (time before injection), 30, 60, and 120 min after venoms or saline injection. Results were calculated as the difference in thickness of both paws, and edema was expressed as the percentage increase in paw thickness.

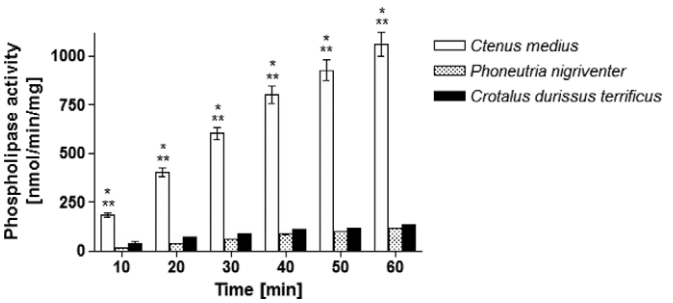

Fig. 2. *Ctenus medius* and *P. nigriventer* venoms phospholipasic activity. Reactions were developed with the substrate 4-nitro-3 (octanoyloxy) benzoic acid in the presence of 10 μg *C. medius* and *P. nigriventer* venoms. The samples were incubated at 37°C for 60 min, and the absorbance (λ414 nm) was read every 10 min. Buffer and snake venom from *C. durissus terrificus* (10 μg) was used as negative and positive controls, respectively. The specific activity of the venoms was expressed as nmol/min/mg venom. Results are representative for three independent experiments and are expressed as the mean of duplicates ± SD. Asterisks show statistically different ($P < 0.05$) values between *C. medius* and *P. nigriventer* (*) and from spider venoms and the positive control (**).

Statistical Analysis. Data were analyzed statistically using the Student's *t*-test. $P < 0.05$ was considered significant.

Results

SDS-PAGE and Western Blot Analysis. The protein profile of *C. medius* venom was analyzed by SDS-PAGE followed by silver staining and compared with *P. nigriventer*. Figure 1A shows that the venoms of the two species differ in composition, number, and intensity of bands. Anti-arachnidic horse serum, produced by the Butantan Institute against a mixture of arthropod venoms, including the one from *P. nigriventer*, and used in Brazil for human serum therapy, could recognize some of the components present in both venoms (Fig. 1B).

Enzymatic Activities of Venoms. To compare the toxic composition of *C. medius* and *P. nigriventer* venoms, the presence of phospholipase, hyaluronidase, and protease activities were measured in in vitro tests. Figure 2 shows that the spiders' venoms contain PLA_2 activity, being what specific activity observed in *C. medius* significantly higher than that for *P. nigriventer*, as well as for the venom control (*Crotalus durissus terrificus*).

The presence of proteolytic activity in *P. nigriventer* and *C. medius* venoms was assayed using a FRET substrate. Table 1 shows that both venoms were able to hydrolyze the peptide, and the venom of *C. medius* was ~20 times more active than the *P. nigriventer* venom. The proteolytic activity detected in *P. nigriventer* was totally inhibited by PMSF, with 0.1 or 1.0 μg of venom, and only partially blocked in *C. medius* venom. Using the metalloproteinase inhibitor, phenanthroline, it was also possible to observe a partial inhibition of the proteolytic activity in both venoms.

Ctenus medius and *P. nigriventer* venoms were also tested for the presence of hyaluronidase activity. Figure 3 shows that they contain high and similar activity, which was significantly superior to that determined for *L. gaucho* spider venom.

Action of Venoms on the Complement System. Figure 4A shows that *C. medius* venom, but not *P. nigriventer* venom, was able to interfere with the complement system under conditions in which classical and alternative pathways of activation were allowed to operate. A consumption of almost 100% of the lytic complement activity of the normal human serum was observed using *C. medius* venom; in the same experimental condition, *P. nigriventer* venom did not cause any consumption of the complement system.

Figure 4B shows that *C. medius* venom is also able to induce partial (0.1 μg) or total cleavage (1 μg) of the α chain of the central component of the comple-

Table 1. Proteolytic activity of *P. nigriventer* and *C. medius* venoms

	P. nigriventer			*C. medius*		
	Control	Phenantroline (5 mM)	PMSF (5 mM)	Control	Phenantroline (5 mM)	PMSF (5 mM)
0.1 μg	164 ± 11	22.5 ± 2.5	NH	3,381 ± 67	2,319 ± 205	714 ± 16
1 μg	254.5 ± 10.5	157.5 ± 17.5	NH	5,586 ± 4	4,809 ± 119	3,127 ± 316

Values are specific activity (UF/min/μg).

Samples of *C. medius* and *P. nigriventer* venoms were mixed with 2 μM of the FRET substrate in PBS at 37°C, in the presence or absence of the protease inhibitors, phenantroline and PMSF. Data presented fit the overall initial velocities measured in the spectrofluorimeter, at each inhibitor concentration with a substrate consumption of <5%. Results are representative for three independent experiments and expressed as the mean of triplicates ± SD.

NH, no hydrolysis detected.

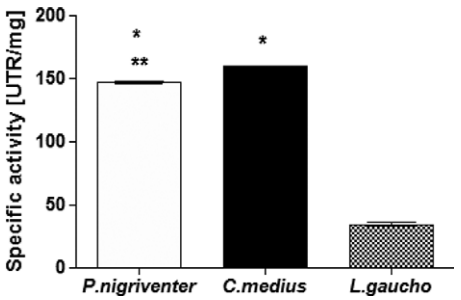

Fig. 3. Hyaluronidase activity of *C. medius* and *P. nigriventer* venoms. Samples of *C. medius* and *P. nigriventer* venoms (10 μg) were mixed with hyaluronic acid substrate, and the reactions were incubated for 15 min at 37°C. The absorbance was measured in a spectrophotometer at λ_{em} = 405 nm, and the hyaluronidase activity was expressed as reduction percentage of turbidity (UTR) of the mixture. The venom from *L. gaucho* was used as positive control. Results are representative for three independent experiments and are expressed as the mean of duplicates ± SD. *Statistically different ($P < 0.05$) values between Ctenidae spider venoms and the positive control (*L. gaucho* venom) and from *C. medius* and *P. nigriventer* venoms (**).

ment system, C3 (9 μg), generating an α'-like fragment. In the same experimental condition, *P. nigriventer* was able to induce partial C3 α chain cleavage only with 1 μg of venom. When, however, PMSF was added into the incubation mixtures, at a final concentration of 5 mM, the C3 cleavage ability of both venoms was completely blocked. The use of phenathroline did not cause any inhibition of the C3 hydrolysis induced by the spiders' venoms.

Hyperalgesia and Edema-inducing Activity. Figure 5A shows that the intraplantar injection of *C. medius* venom, with doses varying from 0.25 to 1.0 μg/paw, evoked a significant decrease in pain threshold (hyperalgesia). Hyperalgesia was detected 30 min after venom injection, decreasing 4 h after treatment and disappearing within 8 h. Except for the dose of 0.1 μg/paw, the maximum responses induced by the distinct venom doses were not statistically different from each other. No effect was found in the saline (control)-injected rats.

The i.pl. injection of *P. nigriventer* venom (1.0 μg/paw) also caused a significant decrease in pain threshold. The peak of the hyperalgesic response was detected 4 h after venom injection (Fig. 5B). The maximum responses induced by *C. medius* (1.0 μg/paw) and *P. nigriventer* venom, although detected at different periods, were not statistically different from each other.

The i.pl. injection of *C. medius* and *P. nigriventer* venoms (1.0 μg/paw) also increased paw volume.

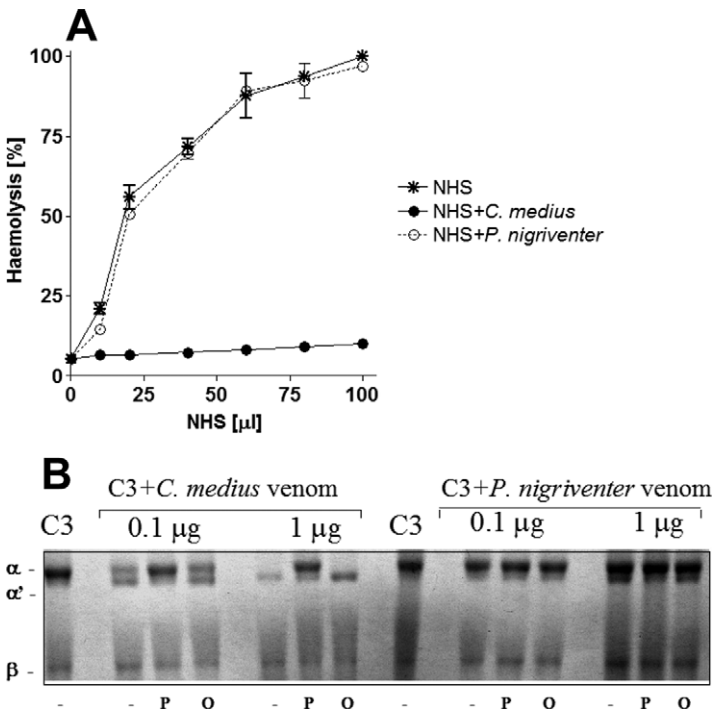

Fig. 4. Action of the spiders' venoms on the complement system. (A) Hemolytic assay. Samples (50 μl) of NHS, as a complement source, were incubated with 50 μg of the spider venoms for 30 min at 37°C. The residual complement lytic activity was measured using antibody-sensitized sheep erythrocytes as a target. After incubation for 1 h at 37°C, the absorbance of the supernatant was measured at λ414 nm and expressed as percentage of lysis. Results are representative for three separate experiments and expressed as mean of duplicates ± SD. (B) Proteolytic action of the spiders venoms on human purified C3. Venoms samples (0.1 or 1 μg) were incubated with the purified component C3 (9 μg) in the presence or absence of 5 mM PMSF (P) or phenathroline (O) for 2 h at 37°C. C3 cleavage was visualized in 10% SDS-PAGE gels, developed under reduction conditions, and stained with Coomassie blue.

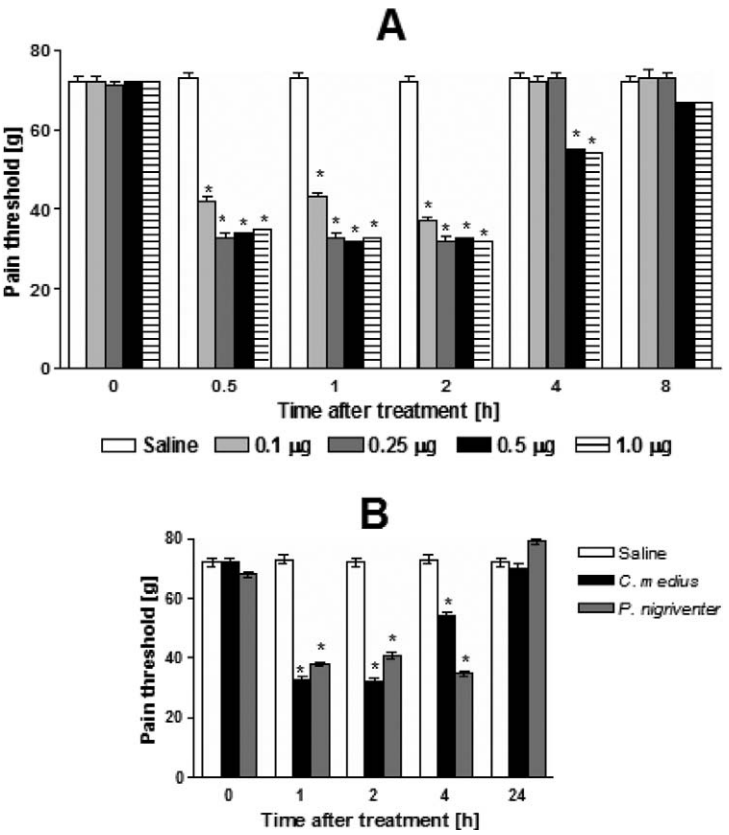

Fig. 5. Comparison of the hyperalgesic effect induced by *C. medius* and *P. nigriventer* venoms. Decrease in pain threshold was determined before (time 0) and at different times after the i.pl. injection of saline in control group (A and B) or of crude venom (1.0 μg/paw) of *C. medius* (A and B) and *P. nigriventer* (B). Sensitivity to pain was measured as the threshold response to pressure (*g*). Each point represents the mean ± SEM for five animals. *Significantly different from mean values before venom injection and of saline group ($P < 0.05$).

Edema was detected 30 min and peaked at 1 h after venom injection, decreasing afterward for *P. nigriventer* venom. For *P. nigriventer* venom, the edematogenic response disappeared within 2 h after injection, whereas for the *C. medius* venom, the edema was still present (Fig. 6).

Discussion

Ctenus medius co-occurs in various microhabitats of the Brazilian Atlantic Forest and can be easily misidentified as the medically important spider *P. nigriventer*. Aiming to verify the toxic potential of the *C. medius* spider venom, some proinflammatory properties were analyzed, including phospholipasic, proteolytic, complement activating, hyperalgesic, and edematogenic activities.

Several components have been isolated from snake venoms including proteases, such as serine proteases, which can act on proteins of the coagulation cascade, inducing platelet aggregation, inducing plasminogen degradation, and increasing vascular permeability (Markland 1998). Using a FRET peptide as substrate for screening the presence of proteolytic activity, it was possible to detect enzymatic activity and to determine the presence of a high content of serine proteinases in *C. medius* and *P. nigriventer* venoms, as shown by the use of PMSF. Using the metalloproteinase inhibitor, phenanthroline, it was also possible to observe a partial inhibition of the proteolytic activity in both venoms. The inhibition of the *P. nigriventer* venom proteolytic activity, by phenanthroline, can be a result of the presence of metal-stabilized serine proteinases in this venom. The partial inhibition of the *C. medius* proteolytic activity, by PMSF and phenanthroline, indicates the presence of more than one class of proteases in this venom.

Activation of complement pathways results in the production of C5a anaphylatoxin, which is a powerful chemoattractant for macrophages and neutrophils and can directly activate these cells to secrete inflammatory mediators (Ember et al. 1998). The membrane attack complex of complement is also implicated in many inflammatory conditions, not so much because it may kill cells, but rather because it causes cell activation, resulting in secretion of chemokines and inflammatory mediators or expression of adhesion molecules (Kilgore et al. 1998).

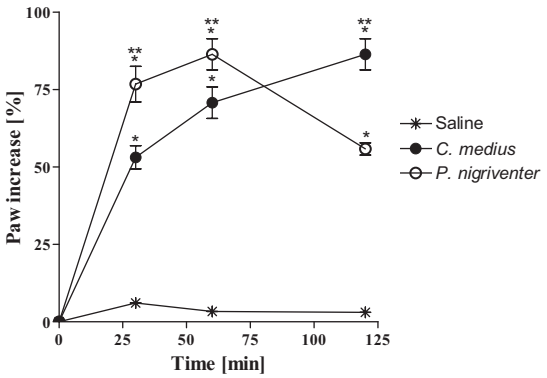

Fig. 6. Comparison of the edematogenic effect induced by *C. medius* and *P. nigriventer* venoms. Edema was induced by i.pl. administration of 0.1 ml sterile saline (0.85% NaCl solution) alone or containing venoms from *C. medius* (1.0 μg) or *P. nigriventer* (1.0 μg) into one of the hind paws. Saline (0.1 ml, control) was injected into the contralateral paw. Paw edema was determined by measuring paw thickness using a caliper at 0 (before treatments), 30, 60, and 120 min after administration of venoms or saline. Results were calculated as the difference in thickness of both paws, and edema was expressed as the percentage increase in paw thickness. *Significantly different from mean values of saline group and **significantly different from mean values of *C. medius* group ($P < 0.05$).

Interestingly, it was observed that serine proteases present in *C. medius* and *P. nigriventer* venoms could induce cleavage of the C3 component of the complement system. Although affecting the central factor of the complement cascades, the venom of *P. nigriventer*, in the conditions used, was not able to interfere in the lytic activity of this system, as shown for the *C. medius* venom. This may be because of the fact that proteolytic activity detected in *C. medius* venom, as measured by the hydrolysis of FRET substrate and purified C3, was much more intense than that exhibited by *P. nigriventer*, which possibly caused a stronger activation and, consequently, a higher consumption of complement components.

Hyaluronidasic activity has been found in the venom of many spiders, both mygalomorphs and araneomorphs. The substrate for hyaluronidase is the mucopolysaccharide, hyaluronic acid, which is a major constituent of the extracellular matrix (Kreil 1995). The presence of hyaluronidase activity was evaluated, and the results showed that *C. medius* and *P. nigriventer* venoms contain similar and higher enzymatic activity than that exhibited by *L. gaucho*, in which the hyaluronidase activity was associated with a 44-kDa protein band (Bárbaro et al. 2004). Components with this molecular mass were detected in both venoms analyzed here, and they may correspond to the hyaluronidases. Further purification studies should solve this aspect.

Venom PLA_2s are divided into three main groups: type I (purified from Elapidae and Hydrophiidae venoms), type II (purified from Crotalidae venoms), and type III (purified from bee, wasp, and the lizard, *Heloderma* spp., venoms). Venom PLA_2s cause local nociceptive (Chacur et al. 2003) and inflammatory effects. The inflammatory response is characterized mainly by increases in vascular permeability (Cirino et al. 1989; Wang and Teng 1990; Landucci et al. 1998, 2000; Chaves et al. 1998) and leukocyte infiltration into tissues (Bomalaski et al. 1991, de Castro et al. 2000). Both nociceptive and inflammatory effects take place by mechanisms dependent and independent of the catalytic activity. Phospholipase A_2 activity was also detected in the venoms of *C. medius* and *P. nigriventer*, and the measured activity for *C. medius* was around eight times higher than that exhibited by *P. nigriventer* and the positive control, i.e., the venom of *C. durissus terrificus.* In *C. durissus terrificus* venom, the PLA_2 formed a complex with an acidic nonenzymatic component named crotapotin, resulting in the Crotoxin complex, the main neurotoxic component, representing ≈50% of the venom protein (Rubsamen et al. 1971, Hendon and Fraenkel-Conrat 1976).

Ctenus medius and *P. nigriventer* venoms were equally able to cause an increased sensitivity to pain (hyperalgesia). Despite showing the same intensity of hyperalgesia, the peak of the hyperalgesic response induced by *C. medius* venom was detected earlier than that of *P. nigriventer* venom. It is important to mention that i.pl. injection of both venoms also caused edema. However, some differences were observed between edematogenic and hyperalgesic responses. Edema peaked at 1 h after venom injection, decreasing thereafter, whereas the maximum hyperalgesic response was detected 2 and 4 h after *C. medius* and *P. nigriventer* venom injection, respectively. In addition, for the *C. medius* venom, edema disappeared 2 h after injection, when the peak of the hyperalgesic effect was being reached. These results indicate that distinct cellular and molecular mechanisms might be involved in the development of both responses and that hyperalgesia is not dependent on the presence of edema. It is important to stress that the edematogenic response of *P. nigriventer* venom is significantly higher than that observed for *C. medius* venom. In conclusion, our data suggest that *C. medius* shares important toxic similarities with *P. nigriventer*, which shows not only an evolutionary convergence of their venom components, but also indicates a potential noxious effect of *C. medius* venom in human accidents.

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