NPC Natural Product Communications

2011
Vol. 6
No. 6
871 - 874

Hypotensive Effects of the *Crotalus durissus cascavella* Venom: Involvement of NO

Janaina S. A. M Evangelista[a], João J. F. Evangelista[b], Inez L.Evangelista[b], Diva M. B. Nojosa[d], Nilberto R. F. Nascimento[a], Marcellus H. L. P.Souza[b], Renata S Alves[c], Alice M C Martins[c,*], Maria Elisabete A. Moraes[b] and Helena S. A. Monteiro[b]

[a]*Veterinary Faculty*, *Estadual University of Ceará*, *Fortaleza*, *Ceará*, *Brazil*
[b]*Department of Physiology and Pharmacology*, *Faculty of Medicine*, *Federal University of Ceará*, *Fortaleza*, *Ceará*, *Brazil*
[c]*Department of Clinical and Toxicological Analysis*, *Federal University of Ceará*, *Fortaleza*, *Ceará*, *Brazil*
[d]*Ophiology Regional Nucleus of Ceará*, *Federal University of Ceara*, *Fortaleza*, *Ceará*, *Brazil*

martinsalice@gmail.com

Received: October 25th, 2010; Accepted: January 14th, 2011

Crotalus durissus cascavella is a snake native of northeastern Brazil. The aim of the study was to investigate the effects of *C. d. cascavella* venom on rat mean arterial pressure and vascular reactivity in the mesenteric vascular bed. The venom evoked a dose-dependent decrease in mean arterial pressure, cardiac and respiratory frequency with increased plasma nitrite levels. L-NAME (10 mg/kg) blunted both the hypotension and increased nitrite production observed after the venom administration. To investigate the effects of *C. d. cascavella* in resistance vessels, the vascular mesenteric bed was studied, and the results suggested that the hypotensive effect of the venom is not dependent on a direct vasodilatory activity. In conclusion, *C. d. cascavella* venom presented indirect hypotensive effects with the involvement of nitric oxide.

Keywords: *Crotalus durissus cascavella* venom, hypotensive effects, blood pressure, mesenteric bed.

Snake venoms are of interest due to their wide range of biologically active substances. Snake venom composition may exhibit variations associated with geographical origin, habitat, seasonal variation, diet, age and gender [1-4].

In Brazil, *Crotalus durissus* species are distributed across five subspecies, *C. d. cascavella, C. d. terrificus*, *C. d. colillineatus*, *C. d. ruruima*, *C. d. marajoensis* and *C. d. cumanensis*. *C. d. cascavella* is an abundant snake in northeastern Brazil [5].

Snake venom contains peptides that exert effects on the vascular system [6]. A hypotensive effect from *Crotalus atrox* venom has been demonstrated [7]. Previously, a novel bradykinin inhibitory peptide in the *Crotalinae* venom family was identified [8]. Recently, Evangelista *et al*. [9] demonstrated the hypotensive effects of a peptide isolated from *Crotalus durissus cascavella* venom. The aim of the present study was to investigate the effects of *Crotalus durissus cascavella* venom on blood pressure and the mesenteric vascular bed.

In the present study, the pressure responses to the whole venom of *Crotalus durissus cascavella* (0.1 and 0.3 µg/Kg) showed a concomitant dose-dependent decrease in mean arterial pressure (control = 100.2 ± 8.1 mmHg; $Cdcasca_{0.1\mu g/Kg}$ = 70.1 ± 6.4* mmHg; $Cdcasca_{0.3\mu g/Kg}$ = 55.3 ± 4.2* mmHg), heart rate (control = 300.2 ± 4.7 beats/min; $Cdcasca_{0.1\mu g/Kg}$ = 200.6 ± 7.8* beats/min; $Cdcasca_{0.3\mu g/Kg}$ = 100.4 ± 3.7* beats/min) and respiratory rate (control = 40 ± 3.6 mL/min; $Cdcasca_{0.1\mu g/Kg}$ = 25 ± 2.9* mL/min; $Cdcasca_{0.3\mu g/Kg}$ = 15 ± 2.1* mL/min) (Figure 1).

The mean arterial pressure increased after L-NAME administration (10 mg/Kg) 30 minutes before the *Crotalus durissus cascavella* venom infusion at all studied doses There was also a significant decrease in arterial pressure of L-NAME treated rats after *Cdcasca* injection (Figure 2).

In the arterial pressure assay, there was an increase in the production of nitrite after infusion of the 0.3 µg/Kg *Crotalus durissus cascavella* venom (Figure 3), with a maximal effect at 30 minutes of the experiment. When the L-NAME was administered 30 minutes before *C. d. cascavella* venom infusion, there was a reduction in nitrite production when compared to the control group (Figure 4)

Previously, Joseph *et al*. [10] demonstrated hypotensive agents derived from snake venoms. In the present study, there was a decrease in the mean arterial pressure, heart

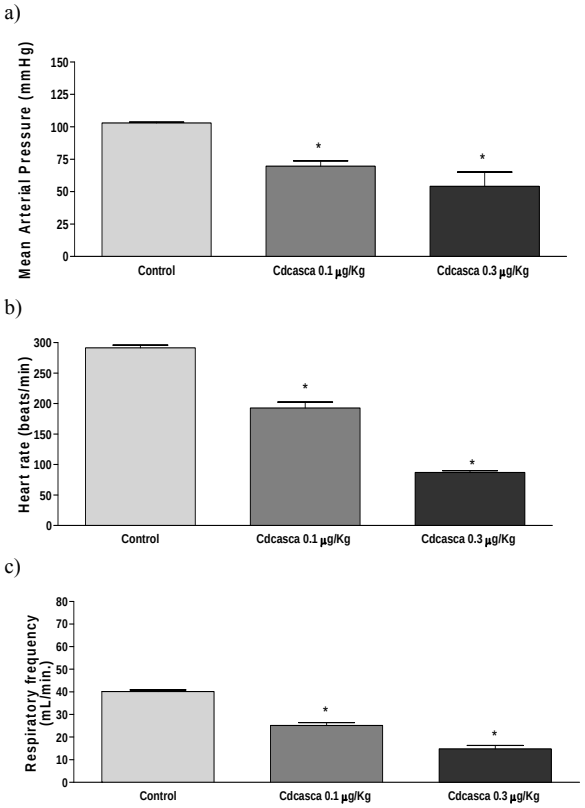

Figure 1: Effects of *Crotalus durissus cascavella* (Cdcasca) venom on mean arterial pressure, heart rate and respiratory frequency. Data are expressed as mean ± SEM from six different animals. *p<0.05 compared to control.

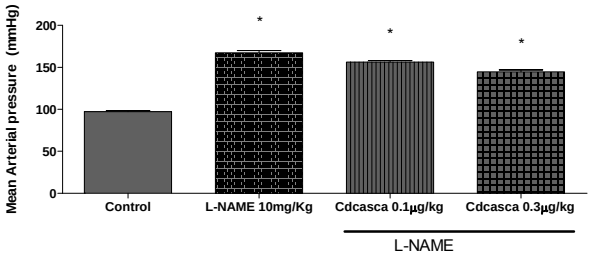

Figure 2: Action of L-NAME on the effects of *Crotalus durissus cascavella* venom in the mean arterial pressure. Data are expressed as mean ± SEM from six different animals. *p<0.05 compared to control.

and respiratory rate after the infusion of *C. d. cascavella* venom. The effect of the venom in arterial pressure and cardiac frequency was acute and presented the same time-course, maybe a phenomenon Bezold-jarisch with vagal great activity. Different mechanisms such as activity on adrenergic or muscarinic receptors [11], activity of chemical mediators in the venom [12] or the release of endogenous autacoids in cardiac tissues [13] have been attributed to chronotropic effects.

The endothelium has an important role in vascular tonus control, and acts as a source of numerous chemical mediators [14,15]. Endothelial cells have a peculiar capacity to secrete and synthesize vasoactive substances that control blood flow, such as nitric oxide, prostacyclin and endothelin [16].

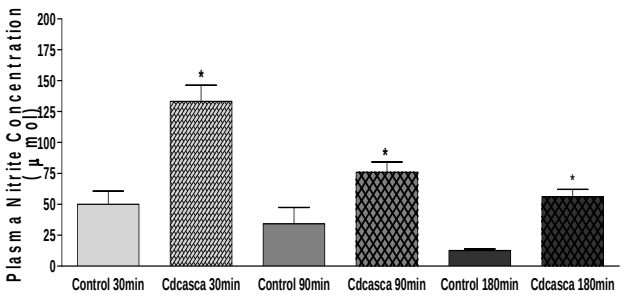

Figure 3: Measurements of plasma nitrite concentration after the infusion of *Crotalus durissus cascavella* venom (0.3µg/Kg). Data are expressed as mean ± S.E.M from six different animals. *p<0.05 compared to the corresponding control group for each interval.

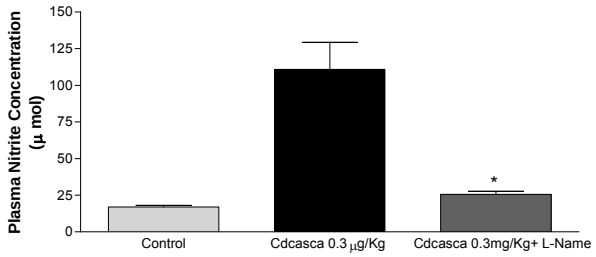

Figure 4: Measurements of plasma nitrite concentration after the pharmacological blockage with L-NAME (10mg/Kg i.p.) 30 min before *Crotalus durissus cascavella* venom infusion. Data are expressed as mean ± S.E.M from six different animals. *p<0.05 compared to control.

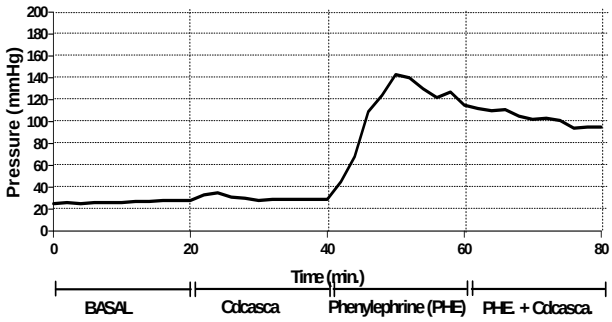

Figure 5: Effects of *Crotalus durissus cascavella* (Cdcasca; (10 µg/mL) venom in mesenteric blood vessels with intact endothelium. Data are expressed as mean ± SEM from six different animals. *p<0.05 compared to the corresponding control group for each interval.

To investigate the effects of *C. d. cascavella* in resistance vessel, the vascular mesenteric bed was studied, which suggested that the action of the venom had no direct vascular effect i*n vitro*. Therefore the hypotensive effect observed *in vivo* may be secondary to either a vagal-mediated cardiovascular reflex, since it is followed by a concomitant decrease in heart rate and to the release of a blood-borne vasoactive substance, such as kinins that would evoke vasodilatation and increase nitrite production. In conclusion, whole *C. d. cascavella* venom presented possible indirect hypotensive effects involving nitric oxide.

Experimental

Blood Pressure Measurements: Male Wistar rats (250-300 g; n=6) were anesthetized with 50 mg/kg of pentobarbital,

the right carotid artery was cannulated with a polyethylene tube (PE50) and the systemic blood pressure was recorded directly through a pressure transducer connected to a 4 channel-polygraph (Narco Biosystems, Houston, Texas, USA). The mean arterial blood pressure was recorded continuously, and after a 30minute equilibration period, the venom and control drugs were injected by a cannula implanted into the jugular vein. The measurements in arterial pressure was recorded immediately after infusion of *Crotalus durissus cascavella* venom (Cdcasca) (0.1 and 0.3 μg/Kg) was injected at 15 min intervals and compared with an isovolumetric injection of saline [17]. The cardiac and respiratory frequencies were obtained by physiographic register and measured by counting the number of cardiac beats and respiratory cycles during a period of one minute respectively.

PAM = PD ± (PS – PD)/3; where PAM is the mean arterial pressure, PS is the systolic pressure and PD is the diastolic pressure.

Nitrite assay: The plasma nitrite (NO^-_2) concentrations were determined by the colorimetric Griess method after the infusion of Cdcasca venom in the blood pressure assay. First, 50 μL of non-diluted samples were incubated with the same volume of Griess reagent (1% sulphanilamide and 0.1% naphthylethylenediamine dihydrochloride in 5% phosphoric acid). A standard nitrite curve was obtained by incubating sodium nitrite (10- 200 μM) with the reductase buffer. The absorbance at 550 nm was determined using a multiwell plate reader (ELx 800 Universal Multiplater). The results were reported as micromolar concentrations (μM) of NO^-_2, comparing the absorbance in samples with the standard curve. In another experiment, the levels of NO^-_2 were studied in groups pre-treated with either saline solution or L-NAME. Then, 30 min before venom infusion, 100 mg/Kg ip of L-NAME (Nitro-L-arginine methyl Ester) was administered and NO^-_2 concentrations were determined as previously described [18].

Isolated perfused arteriolar mesenteric bed: The perfusion was performed following the descriptions of McGregor [19]. Briefly, Wistar rats weighing 280-350 g were anesthetized with sodium pentobarbital (50 mg/Kg, b.w.).After opening the abdomen, the pancreatic-duodenal, ileum-colic and colic branches of the superior mesenteric artery were tied. Then, the superior mesenteric artery was cleaned of surrounding tissue and cannulated with a polyethylene tube (PE20). The intestine was separated from the mesenteric bed by cutting close to the intestinal border of the mesentery. The mesenteric bed was perfused with Krebs solution containing: 114.0 mM of NaCl; 4.96 mM of KCl; 1.24 mM of KH_2PO_4; 0.5 mM of $MgSO_4.7H_2O$; 24.99 mM of $NaHCO_3$; 2.10 mM of $CaCl_2.2H_2O$; and 3.60 mM of glucose. The perfusion solution was kept at 37ºC and the mesenteric bed was perfused with a constant flow (4 mL/min), while the variable perfusion pressure was measured by means of a pressure transducer (Statham P23, Gould, Oxnard, CA, USA) connected to the arm side of the system. The variations in perfusion pressure were continuously recorded on a four-channel physiograph (Narco BioSystems, Houston, TX, USA). In the set up, the direct vascular effects of *Crotalus durissus cascavella* venom (10μg/mL/min; n=6), infused for 10 min at a constant rate (0.1 mL/min), were examined and compared to the infusion of the vehicle alone at the same rate. The effects of *C. d. cascavella* on perfusion pressure were further compared with the pressor effect attained with a 1μM/mL/min phenylephrine infusion. Additionally, 1μM acetylcholine was injected in bolus (in the plateau-phase of phenylephrine-induced contraction) to confirm endothelium functional integrity.

Statistical analysis: The data are presented as mean ± SEM. The means were evaluated by ANOVA followed by Dunnet's test, when appropriated. Values of $p<0.05$ were considered statistically significant. Prism version 5.0 was used for all statistics.

Ethic Aspects: The study protocol was approved by Ethics Committees from the Federal University of Ceará, in Fortaleza, Brazil (nº 107/07).

Acknowledgements - Financial support from CNPq and FUNCAP.

References

[1] Chippaux JP, Boussinesq M, Ranque S, Schneider D. (**1998**) Occurrence of onchocerciasis in subjects coming from non-endemic areas and migrating to a hyperendemic area. *Bulletin de la Société de pathologie exotique*, ***91***, 173-174.

[2] Mackessy SP, Williams K, Ashton KG. (**2003**) Ontogenetic Variation in Venom Composition and Diet of *Crotalus oreganus concolor*: A Case of Venom Paedomorphosis. *Copeia*, ***4***, 769-782.

[3] Furtado MF, Travaglia-Cardoso SR, Rocha MM. (**2006**) Sexual dimorphism in venom of *Bothrops jararaca* (Serpentes: *Viperidae*). *Toxicon*, ***48***, 401-10.

[4] Queiroz GP, Pessoa LA, Portaro FC, Furtado MDEF, Tambourgi D. (**2008**) Interspecific variation in venom composition and toxicity of Brazilian snakes from *Bothrops* genus. *Toxicon*, ***52***, 842-851.

[5] Pinho FM, Pereira ID. (**2001**) Snake bites. *Revista da Associação Médica Brasileira*, ***47***, 24-29.

[6] Yamazaki Y, Morita T. (**2007**) Snake venom components affecting blood coagulation and the vascular system: structural similarities and marked diversity. *Current pharmaceutical design*, ***13***, 2872-2886.

[7] de Mesquita LC, Selistre HS, Giglio JR. (**1991**) The hypotensive activity of *Crotalus atrox* (western diamondback rattlesnake) venom: identification of its origin. *American Society of Tropical Medicine and Hygiene*, ***44***, 345-353.

[8] Graham RL, Graham C, Mcclean S, Chen T, O´Rourke M, Hirst D, Theakston D, Shaw C. (**2005**) Identification and functional analysis of a novel bradykinin inhibitory peptide in the venoms of New World *Crotalinae* pit vipers. *Biochemical and Biophysical Research Communications*, ***338***, 1587–1592.

[9] Evangelista JS, Martins AM, Nascimento NR, Sousa CM, Alves RS, Toyama DO, Toyama MH, Evangelista JJ, Menezes DB, Fonteles MC, Moraes ME, Monteiro HS. (**2008**) Renal and vascular effects of the natriuretic peptide isolated from *Crotalus durissus cascavella v*enom. *Toxicon*, ***52***, 737-744.

[10] Joseph R, Pahari S, Hodgson WC, Kini RM. (**2004**) Hypotensive agents from snake venoms. *Current Drug Targets Cardiovascular & Hematological Disorders*, ***4***, 437-459.

[11] Church JE, Hodgson WC. (**2002**) Adrenergic and cholinergic activity contributes to the cardiovascular effects of lionfish (*Pterois volitans*) venom.*Toxicon*, ***40***, 787–796

[12] Hopkins BJ, Hodgson WC, Sutherland SK. (**1996**) Evidence for adrenergic and tachykinin activity in venom of the stonefish (Synanceja trachynis). *Toxicon*, ***34*** , 541-554.

[13] Hopkins BJ, Hodgson WC. (**1998**) Cardiovascular studies on venom from the soldierfish (*Gymnapistes marmoratus*). *Toxicon*, ***36***, 973–983.

[14] Griffith TM. (**2002**) Endothelial control of vascular tone by nitric oxide and gap junctions: A haemodynamic perspective. *Biorheology*, ***39***, 307-318.

[15] Sabrane K, Kruse MN, Fabritz L, Zetsche B, Mitko D, Skryabin BV, Zwiener M, Baba HA, Yanagisawa M, Kuhn M. (**2005**) Vascular endothelium is critically involved in the hypotensive and hypovolemic actions of atrial natriuretic peptide. *The Journal of Clinical Investigation*, ***115***, 1666-1674.

[16] Flora Filho R, Zilberstein B. (**2000**) Nitric oxide: the simple messenger passing through complexity. Metabolism, synthesis and functions. *Revista da Associação Médica Brasileira*, ***46***, 265-271.

[17] Chen KK, Kovaríková A. (**1967**) Pharmacology and toxicology of toad venom. *Journal of Pharmaceutical Sciences*, ***56***, 1535-1541.

[18] Sun J, Zhang X, Broderick M, Fein H. (**2003**) Measurement of Nitric Oxide Production in Biological Systems by Using Griess Reaction Assay. *Sensors*, ***3***, 276-284.

[19] McGregor DD. (**1965**) The effect of sympathetic nerve stimulation of vasoconstrictor responses in perfused mesenteric blood vessels of the rat. *The Journal of Physiology*, ***177***, 21-30.