Cardiovascular activity of *Micrurus lemniscatus lemniscatus* (South American coralsnake) venom

Rafael S. Floriano[a,b], Kristian A. Torres-Bonilla[b], Julio A. Rojas-Moscoso[b], Lourdes Dias[b], Thalita Rocha[c], Nelson Jorge da Silva Jr.[d], Stephen Hyslop[b], Edward G. Rowan[e*]

[a]Graduate Program in Health Sciences, University of Western São Paulo (UNOESTE), Rodovia Raposo Tavares, km 572, B2-208, 19067-175, Presidente Prudente, SP, Brazil

[b]Department of Pharmacology, Faculty of Medical Sciences, State University of Campinas (UNICAMP), Rua Tessália Vieira de Camargo, 126, 13083-887, Campinas, SP, Brazil

[c]Multidisciplinar Research Laboratory, São Francisco University (USF), Av. São Francisco de Assis, 218, 12916-900, Bragança Paulista, SP, Brazil

[d]Graduate Program in Environmental Sciences and Health, School of Medical, Pharmaceutical and Biomedical Sciences, Pontifical Catholic University (PUC Goiás), Rua 232, 128, 74605-140, Goiânia, GO, Brazil

[e]Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Cathedral Street, 161, G4 0RE, Glasgow, UK

Running title: Cardiovascular activity of *M. l. lemniscatus* venom

*Corresponding author: E.G. Rowan (e.g.rowan@strath.ac.uk), Tel.: +44 141 548 2689, Fax: +44 141 552 2562

Abstract

Envenomation by coralsnakes (*Micrurus* spp.) is characterized by blockade of peripheral neurotransmission mediated by the presence of α- and β-neurotoxins. However, little is known about their cardiovascular activity. *Micrurus lemniscatus lemniscatus* is a coralsnake found in the Amazon basin and occasionally causes envenomation in humans. In this study,

we examined the hemodynamic, vascular and atrial responses to *M. l. lemniscatus* venom. Anesthetized rats were used for hemodynamic and electrocardiogram (ECG) recordings; in vitro experiments were carried out in rat isolated thoracic aorta and atria preparations. In vivo, venom (0.1 and 0.3 mg/kg) caused immediate and persistent hypotension that was maximal within the first minute with both doses being lethal after ~40 and ~20 min, respectively. ECG, heart and respiratory rates were not altered during the transient hypotension phase induced by venom but all altered prior to death. There was no evidence of myonecrosis in cardiac muscle tissue, pulmonary hemorrhage nor thrombosis in anesthetized rats exposed to venom. In vitro, venom (10 μg/ml) did not contract aortic strips nor affected the maximal responses to pre-contraction with phenylephrine (PE, 0.0001–30 μM) in strips with and without endothelium. However, venom (10 μg/ml) relaxed aortic strips with endothelium pre-contracted with PE. In aortic strips pre-contracted with PE, venom prevented acetylcholine (0.0001–30 μM)-induced relaxation in strips with endothelium without affecting relaxation induced by sodium nitroprusside (0.1–100 nM) in strips without endothelium. Venom (30 μg/ml) produced a transient increase of atrial contractile force without affecting atrial rate. Taken together these findings indicate a predominantly vascular action of the venom, most likely involving toxins interacting with muscarinic receptors.

Keywords: *M. l. lemniscatus*, coralsnake venom, hemodynamic, cardiotoxicity, vascular reactivity, endothelial action.

1. Introduction

Coralsnakes (*Micrurus* spp.) are represented by more than 80 species widely distributed throughout the Americas (Campbell and Lamar, 2004; Roze, 1996; Silva Jr. et al., 2016). In Brazil, there are approximately 34 species distributed around the country including *Micrurus*

lemniscatus, with three recognized subspecies: *M. l. carvalhoi* is distributed along the Brazilian east coast from the northeast to southeast and central west of the country, *M. l. helleri* occurs in the western Brazilian Amazon and *M. l. lemniscatus* found in the central Brazilian Amazon and northern Brazil (Costa and Bérnils, 2018; Floriano et al., 2019; Nogueira et al., 2019; Silva Jr. et al., 2016). Despite this large distribution, human envenomation by coralsnakes correspond to ~1% of the snakebites registered in Brazil and only a few species are involved in these reports (Bisneto et al., 2020; Bucaretchi et al., 2016a,b; Melgarejo et al., 2016; Risk et al., 2016; Rodrigo et al., 2016; Silva et al., 2018; Souza et al., 2016; Strauch et al., 2018) with *Micrurus lemniscatus* being the third most frequent species involved (Bucaretchi et al., 2016b).

Several biological activities have been described for *Micrurus* venoms, such as intense pain (Nishioka et al., 1993; Vital Brazil et al., 1976/1977; Vital Brazil and Vieira, 1996), edema (Cecchini et al., 2005; Gutiérrez et al., 1980; Moraes et al., 2003; Urdaneta et al., 2005), myonecrosis (Arroyo et al., 1987; Barros et al., 1994; Gutiérrez et al., 1992; Gutiérrez et al., 1986; Moraes et al., 2003), haemorrhage (Barros et al., 1994; Francis et al., 1997; Ramsey et al., 1972), nephrotoxicity (Braga et al., 2020; De Roodt et al., 2012) and interference with the complement system (Tanaka et al., 2012). However, the peripheral neurotoxicity is the most relevant clinical manifestation of envenomation by coralsnakes and it represents the principal cause of death due to rapid neuromuscular blockade (Bucaretchi et al., 2016a,b; Floriano et al., 2019; Risk et al., 2016; Warrell, 2004). The neurotoxicity of *Micrurus* venoms is mediated by two major groups of toxins: three-finger toxins (3FTx), classic α-neurotoxins that block post-synaptic nicotinic (cholinergic) receptors, and a variety of phospholipase A_2 (PLA_2), some of which act as β-neurotoxins causing potent presynaptic blockade of neurotransmitter release (Aird et al., 2017; Bénard-Valle et al., 2020; Bucaretchi et al., 2016a,b; Gutiérrez et al., 2016; Lomonte et al., 2016; Schütter et al., 2019).

In comparison to the well-studied neurotoxic effects of *Micrurus* venoms, the cardiotoxic and vascular effects of these venoms have been poorly investigated with only a relatively small number of studies describing the hypotensive effects of coralsnakes venoms in the 1970s (Ramsey et al., 1971; Ramsey et al., 1972; Vital Brazil et al., 1976/1977; Weiss and McIsaac, 1971). In this study, we have examined the activity of *Micrurus l. lemniscatus* venom on the arterial pressure, atrial activity and vascular reactivity using hemodynamic monitoring in anesthetized rats and myographical approaches in rat isolated atria and thoracic aorta preparations with the aim of further enhancing our knowledge of the physiological consequences of being envenomed by these important snake species.

2. Material and methods

2.1. Reagents and venom

All salts for the physiological solutions were of analytical grade. *Micrurus l. lemniscatus* venom was obtained from adult snakes of both sexes captured around the Belo Monte hydroelectric power dam at Altamira in the northern Brazilian state of Pará in the Brazilian Amazon (Brazilian Institute of the Environment and Renewable Natural Resources–IBAMA, collecting permits nos. IBAMA-ABIO 647/2015 and IBAMA-ABIO 983/2018); the snakes were identified by a professional herpetologist with extensive experience in coralsnake taxonomy (N. J. Silva Jr.). The venom was desiccated and stored at -20 °C until used. This study was registered with the Brazilian National System for the Management of Genetic Patrimony and Associated Traditional Knowledge (SISGEN, registration no. A93A904).

2.2. Animals

Wistar rats (350–450 g) obtained from the Multidisciplinary Center for Biological Investigation (CEMIB/Unicamp) were housed in plastic cages (3 animals/cage) with a wood-

shaving substrate, at 23 °C in ventilated stands (Alesco®) on a 12 h light/dark cycle with lights on at 6 a.m. The animals had free access to food and water. For in vitro experiments, rats were euthanized with isoflurano (saturated atmosphere) immediately prior to the experiments. For in vivo experiments, rats were maintained under constant sedation by isoflurane (2% in air) during the hemodynamic protocols and then euthanized by slowly increasing the concentration of isoflurane at the end of the experiment. All experimental protocols were carreid out according to the guidelines of the Brazilian College for Animal Experimentation (COBEA) and approved by the institutional Committee for Ethics in Animal Use (CEUA/UNICAMP, protocol no. 4913-1/2018).

2.3. Hemodynamic measurements in anesthetized rats

Male Wistar rats were anesthetized with isoflurane (induction with 3%/l/min and maintained at 1.5%/l/min in 98.5% O_2), the left carotid artery was dissected and exposed, followed by catheterization with heparinized polyethylene cannula (10 U/ml) for systemic arterial pressure measurement, as essentially described elsewhere (Dias et al., 2016). The left femoral vein was also surgically exposed, followed by catheterization with heparinized polyethylene cannula to inject venom at doses of 0.1 and 0.3 mg/kg (in 100 μl of 0.9% saline); the doses of venom (0.1 and 0.3 mg/kg) were chosen based on preliminary experiments which showed that doses < 0.03 mg/kg produce minimal hemodynamic alterations whereas doses > 0.3 mg/kg cause rapid death. Cardiorespiratory parameters were monitored, continuously, through a modular multiparameter monitor (PowerLab, ADInstruments Pty Ltd., New South Wales, Australia) and analyzed using a software Labchart Pro v.7 (ADInstruments), which included: 1) arterial blood pressure (systolic, diastolic, mean and pulse pressures) recorded through a model MLT-0699/670 blood pressure transducer (ADInstruments), 2) heart rate (HR) and 3) electrocardiogram (ECG), both

recorded through surface electrodes (D2 derivative). ECG was monitored through electrodes positioned on the plantar surface of the left and right front paws and right hind paw and coupled to a model MLS-360 Animal BioAmplifier. The heart rate and cardiac arrhythmias were determined from ECG recordings. Respiratory rate was determined manually. The cardiorespiratory parameters were monitored up to 120 min and surviving rats were killed with an overdose of anesthetic. Samples of heart and lungs tissues were collected and processed for histological analysis.

2.4. Rat isolated thoracic aorta preparation

The direct action of the venom on vasculature was assessed in rat isolated thoracic aorta. Male rats were euthanized with isoflurane (saturated atmosphere) and then exsanguinated to dissect the thoracic portion of the aorta artery which was maintained in Krebs solution (composition, in mM: NaCl 118, KCl 4.7, $NaHCO_3$ 14.9, KH_2PO_4 1.2, $MgSO_4 \cdot 7H_2O$ 1.17, $CaCl_2$ 2.5 and glucose 5.6, pH 7.4) aerated with carbogen (95% O_2–5% CO_2) at 37 ºC. The vessel's surface connective tissue was carefully removed and sectioned in strips of approximately 2 mm in length, preserving the endothelium in some of them (E^+) and in other strips removing it by mechanical debridement (E^-). The strips were subsequently mounted in a 5 ml organ bath containing aerated (carbogen) Krebs solution. Initially, the strips were subjected to a 10 mN tension (considered an ideal tension; the same tension was applied to all experiments) using a force transducer (Ugo Basile, Varese, Italy) connected to a PowerLab 4/35 data-acquisition system (Software LabChart, version 7.3.7; ADInstruments, Colorado Springs, MA, USA). In order to verify their contractile viability, the strips were individually exposed to KCl (80 mM), a membrane depolarizing agent, to induce a maximal contraction (considered 100% of response); in some protocols, these values were used to normalise the contractile responses to phenylephrine (PE), an α1-adrenergic agonist.

Subsequently, exogenous acetylcholine (ACh, 3 µM), a muscarinic agonist, was used to determine the physiological status of the endothelium in strips pre-contracted with phenylephrine (3 µM); the absence of endothelium was confirmed by loss of relaxing response (relaxation < 20%) to exogenous ACh (3 µM); only strips showing relaxation > 70% were considered to have functionally preserved endothelium. After these controls, the strips were extensively washed and maintained under basal tension for 60 min prior to the start of the experiment. The strips (E^+ or E^-) were then contracted by PE (0.0001–30 µM), in order to obtain a concentration-response curve. The strips were washed and then incubated with 10 µg of venom/ml [concentration chosen based on its capacity to induce complete neuromuscular blockade in vitro on mouse phrenic nerve diaphragm preparation (Floriano et al. 2019] for 30 min [time required to induce complete neuromuscular blockade in mouse phrenic nerve diaphragm preparation (Floriano et al., 2019)] to assess its capacity to induce direct contracture. In the absence of direct contracture activity, the strips were post-contracted with PE (0.0001–30 µM) to assess the integrity of the contracture function by α1-adrenergic agonist. Some strips (E^+ and E^-) were previously exposed to venom (10 µg/ml) and then pre-contracted with PE (3 µM); followed by, sodium nitroprusside (SNP, 0.1–100 nM) or ACh (0.0001–30 µM).

2.5. Rat isolated atrial preparations

Male rats were euthanized with isoflurane (saturated atmosphere) and exsanguinated subsequently; the heart was removed and maintained in modified Krebs–Henseleit (KH) solution (composition in mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 0.45, $NaHCO_3$ 25, KH_2PO_4 1.03, D-glucose 11.1 and ascorbic acid 0.14, pH 7.4), as essentially described elsewhere (Rodrigues et al., 2014). The right and left atria were dissected and mounted under a tension of 1 g in an organ bath (Panlab, Barcelona, Spain) containing 5 ml of KH solution at

37 °C and aerated with carbogen (95% O_2–5% CO_2). The left atrium was positioned in double rings type bipolar electrodes and subjected to supramaximal stimulation (LE 12406 TC stimulator, Panlab, Barcelona, Spain) with rate of stimulus adjusted according to the rate of spontaneous twitches seen with the right atrium (~5 Hz; 2 ms; ~5V). The atrial rate and contractile force of spontaneous and evoked twitches from right and left atria, respectively, were recorded continuously by a TRI201AD force displacement transducer coupled to a Quad Bridge Amp and computer configured with a software LabChart 6.0 software (ADInstruments). After stabilization for 20 min, the atria were washed three times with KH solution and, 20 min later, venom (30 µg/ml) [maximal concentration to induce maximum neuromuscular blockade in vitro on mouse phrenic nerve diaphragm preparation (Floriano et al., 2019)] was added directly into the organ bath; control preparations were maintained in KH solution without venom. The alterations in atrial rate and contractile force were monitored during 60 min. At the end of the experiments, the atria were processed for histological analysis.

2.6. Morphological analysis

The samples of heart and lungs from those animals used for hemodynamic experiments as well as atria from animals used for in vitro protocols were fixed in 10 % formaldehyde, dehydrated ethanol, clarified in xylol and embedded in paraffin. Serial sections, 5 µm thick, were obtained using a Lupetec MRP2015 microtome. The sections were stained with hematoxylin-eosin (HE) and examined with a Leica DFC 300FX CCD light microscope coupled to a computer running Q Win Plus v. 3.2.0.

203 *2.7. Statistical analysis*

204 In experiments with rat isolated thoracic aorta preparation, relaxations and contractions
205 were measure relative to the maximum contraction produced by PE and the pEC50 was
206 calculated using a sigmoidal model -*log* of the concentration vs. functional responses. The
207 results were expressed as the mean ± SEM and statistical comparisons were done using
208 Student's *t*-test or ANOVA followed by the Tukey-Kramer test. A value of $p < 0.001$, $p <$
209 0.01 and $p < 0.05$ indicated significance. All data were analyzed using Microcal Origin 8 SR4
210 v. 8.0951 (Microcal Software Inc., Northampton, MA, USA) and GraphPad Prism 4 v. 4.03
211 (GraphPad Software Inc., La Jolla, CA, USA) software.
212
213 **3. Results**

214 In anesthetized rats, *M. l. lemniscatus* venom (i.v.) caused immediate and significant
215 decrease in arterial blood pressure; mean arterial pressure remained significantly low with a
216 tendency to return to normal after approximately 10 min, followed by cardiovascular collapse
217 characterized by heart and respiratory failure, resulting in death after ~40 and ~20 min for
218 doses of 0.1 and 0.3 mg of venom/kg, respectively (Figure 1A). Both of systolic and diastolic
219 blood pressure measurements were affected by venom (Figure 1B-C) with no significant
220 alteration of pulse pressure monitored during the hypotension phase (Figure 1D). Fig. 2A
221 shows a representative recording of the blood pressure responses to *M. l. lemniscatus* venom
222 (0.1 mg/kg; i.v.) in anesthetized rats; heart and respiratory rates were not affected during the
223 hypotension phase seen with both of venom doses (Figure 2B-C). The histological analysis of
224 the heart and lung samples revealed no structural or cellular alterations due to i.v. injection of
225 venom (Figure 3A-B).

226 In rat thoracic aorta strips, *M. l. lemniscatus* venom did not induce significant
227 alterations on the vasoconstriction curve by phenylephrine (PE) after incubation with venom

in strips with (E^+) and without (E^-) endothelium [(E^+) Emax: 4.02 ± 0.86 (pre-) vs. 2.73 ± 0.67 (post-incubation), pEC50: 7.02 ± 0.17 (pre-) vs. 6.91 ± 0.21 (post-incubation); (E^-) Emax: 10.41 ± 2.21 (pre-) vs. 7.06 ± 1.27 (post-incubation), pEC50: 7.74 ± 0.20 (pre-) vs. 7.18 ± 0.16 (post-incubation); $p > 0.05$, n = 6] (Figure 4A). In strips E^- pre-contracted with PE, sodium nitroprusside (SNP) produced concentration-dependent vascular relaxation; the pre-incubation of strips with 10 μg of venom/ml did not affect the vascular relaxation promoted by SNP [(E^-) Emax: 94.71 ± 6.80 (pre-) vs. 99.89 ± 1.18 (post-incubation), pEC50: 8.55 ± 0.10 (pre-) vs. 8.82 ± 0.05 (post-incubation); $p > 0.05$, n = 6] (Figure 4B). In strips E^+ pre-contracted with PE, ACh produced concentration-dependent vascular relaxation; however, in strips pre-incubated with venom (10 μg/ml), the vascular relaxation induced by ACh was significantly prevented [(E^+) Emax: 99.45 ± 2.74 (pre-) vs. 22.49 ± 4.23* (post-incubation), pEC50: 7.08 ± 0.07 (pre-) vs. 6.16 ± $0.18^{\#}$ (post-incubation); $^*p < 0.001$ and $^{\#}p < 0.01$ compared to pre-venom incubation curve (n = 6)] (Figure 4C). In addition, *M. l. lemniscatus* venom (10 μg/ml) produced significant vascular relaxation in strips E^+ pre-contracted with PE [% of relaxation: 67.29 ± 8.11* (venom) vs. 13.27 ± 1.75 (vehicle); $^*p < 0{,}001$ compared to strips exposed to vehicle (saline), n = 6)] (Figure 4D).

In rat isolated atria preparations, *M. l. lemniscatus* venom (30 μg/ml) caused a transient and significant increase in the contractile force recorded from right atrium between 10- and 20-min incubation, progressing to basal values in amplitude after 30 min. In directly stimulated left atrium, the increase seen in the contractile force caused by venom was maximal after 10 min and remained significantly elevated until the end of the incubation (60 min) (Figure 5A) period. Venom did not alter the evocated and spontaneous atrial rate (Figure 5B).

4. Discussion

The results of this study provide a general view of the cardiovascular action of *Micrurus lemniscatus lemniscatus*, an Amazonian coralsnake, venom on the hemodynamic parameters in anesthetized rats, including its effects on the vascular reactivity and atria contractility in vitro. To date, *M. l .lemniscatus* is the third coralsnake species for which there are now information about the cardiovascular effects produced by its venom, with the other two species being *M. fulvius* (Ramsey et al., 1971; Ramsey et al., 1972; Weiss and McIsaac, 1971) and *M. frontalis* (Francis et al., 1998; Vital-Brazil et al., 1976/1977) whose venoms were studied mainly in the 1970s.

Here, we have observed that *M. l. lemniscatus* venom at doses of 0.1 and 0.3 mg/kg (i.v.) produced an immediate and persistent hypotension followed by death after ~40 and ~20 min, respectively, in anesthetized rats. During the transient hypotension phase, there were no significant alterations to the ECG parameters, heart and respiratory rate, including absence of morphological changes to heart and lung samples. Comparably, Weiss and McIsaac (1971) reported that an i.v. infusion of *M. fulvius* venom (2 mg/kg during 60 min) in anesthetized cats reduced (~30%) arterial pressure followed by slight recovery and progressive failure after 120 min; *M. fulvius* venom did not cause alterations in heart rate or ECG during the hypotension phase although there was evidence of myocardium injury. In addition, *M. fulvius* venom (0.3 and 0.5 mg/kg, i.v.) caused a reduction of cardiac output, aortic pressure, increased pulmonary arterial pressure, systemic vascular resistance and hepatic pressure in anesthetized dogs; *M. fulvius* venom also produced an initial decrease in the heart rate and increase in the myocardium contractility with a reduction in both parameters in the final phase (Ramsey et al., 1972). The direct cardiac action of *M. fulvius* venom in rats (Ramsey et al., 1971) and its capacity to depolarize rat skeletal muscle membrane (Weiss and McIsaac, 1971)

would suggest the presence of compounds with similar mechanism of action to cardiotoxins, however, *Micrurus* venoms do not contain cardiotoxins (Aird et al., 2017).

Vital Brazil et al. (1976/1977) reported that an i.v. injection of *M. frontalis* venom in anesthetized dogs caused a pronounced decrease in arterial pressure, followed by heart attack that resulted in death; however, it was possible to reverse the cardiovascular collapse through cardiac massage, artificial ventilation and administration of norepinephrine. The authors also showed that in animals exposed to venom by i.m. injection, the decrease in arterial pressure was considerably lower compared to i.v. administration, with death resulting from respiratory paralysis rather than cardiovascular collapse; with both routes of venom-administration, there was no alteration in ECG. These series of experiments performed by Vital Brazil et al. (1976/1977) showed that the route of venom-administration can determine the cause of the death, with i.v. injection inducing severe hypotension followed by heart and respiratory failure whereas by i.m. injection the animal progress to respiratory paralysis. In addition, the artificial ventilation used in order to increase the survival of dogs exposed to *M. frontalis* venom showed to be ineffective in cats (Weiss and McIsaac, 1971) and dogs (Ramsey et al., 1972) exposed to *M. fulvius* venom. Some years later, Francis et al. (1998) also reported that a sublethal dose (0.5 μg/g) of *M. frontalis* venom reduced blood pressure (~40%) within 5–10 min in mice after i.v. injection, with no alteration in heart rate.

We have also examined the effect of *M. l. lemniscatus* venom on the vascular smooth muscle contractile response induced by an α1-adrenergic agonist, phenylephrine (PE), in rat thoracic aorta strips with (E^+) and without (E^-) endothelium, and there was no significant alteration of the vasoconstriction curve. In addition, this venom does not seem to affect vascular smooth muscle function since sodium nitroprusside, an endothelium independent nitric oxide donor drug, produced vascular relaxation in strips E^- pre-contracted with PE in tissues previously exposed to venom. However, the attenuation of the ACh-induced vascular

relaxation by *M. l. lemniscatus* venom and the significant vascular relaxation produced by venom both in strips E^+ pre-contracted with PE, suggesting the presence of substances affecting the function of the vascular muscarinic receptors. Fig. 6 summarizes these pharmacological strategies carried out in rat aorta thoracic preparation, including the signalling pathways involved in the process of vascular contraction and relaxation. Two muscarinic toxins have been characterized from a very closely related species of *Micrurus* (= *M. lemniscatus carvalhoi*), being a peptide 'MT-Mlα' (Coelho da Silva et al., 2011) and a PLA_2 'Mlx-8' (Dos Santos et al., 2020) which affecting muscarinic receptors in rat hippocampus similarly to atropine and pirezenpine. Venom did not cause alterations in the rate of paced or spontaneously active atria in vitro; despite venom increasing contractile force in rat isolated atria preparations, there were no changes cardiac function as examined in anesthetized rats during the hypotension phase induced by venom.

The hypotension induced by *M. l. lemniscatus* venom seems to involve predominantly a vascular mechanism of action, without affecting directly the cardiac functions; the relaxation induced by venom in rat thoracic aorta preparation strongly suggests that the primary site of action is vascular. The mechanisms of action of *M. l. lemniscatus* venom on the vasculature still need detailed investigated in order to better understand the consequences of human envenomation. With the recent advances in proteomic, transcriptomic and genomic approaches (Aird et al., 2017; Bénard-Valle et al., 2020; Ciscotto et al., 2011; Corrêa-Netto et al., 2011; Fernández et al., 2011, 2015; Lippa et al., 2019; Lomonte et al., 2016; Olamendi-Portugal et al., 2018; Rey-Suárez et al., 2011, 2016; Sanz et al., 2016, 2019), the venom from *M. l. lemniscatus* may have biotechnological applications e.g. pharmacological tools and/or new therapeutic agents/scaffolds for the treatment of hypertension. In conclusion, the lack of effect of *M. l. lemniscatus* venom on the cardiac (ECG and heart rate) and respiratory parameters, as well as on heart and lung morphology, suggest that the venom-induced

hypotension is likely via a vascular mechanism of action. The hypotension caused by *M. l. lemniscatus* venom in anesthetized rats and the venom-induced relaxation in rat isolated thoracic aorta preparation indicate the presence of toxins acting on endothelial muscarinic receptors.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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535

536 **Legends**

537 Figure 1. Hemodynamic responses to *M. l. lemniscatus* venom (0.1 and 0.3 mg/kg) in
538 anesthetized rats. Effects induced by venom on the mean arterial pressure (A), systolic blood
539 pressure (B), diastolic blood pressure (C), and pulse pressure (D). In control experiments,
540 saline (0.9%, 100 μl) was administered intravenously (i.v.) via a femoral vein. The points
541 represent the mean ± SEM; (n = 5); *$p < 0.05$ compared to basal values, $^{\#}p < 0.05$ compared
542 to saline control group.
543

544 Figure 2. Representative recording of the blood pressure responses to *M. l. lemniscatus*
545 venom (0.1 mg/kg) (A) and effects induced by venom on the heart (B) and respiratory (C)
546 rates in anesthetized rats. In B and C, the points represent the mean ± SEM; (n = 5); *$p < 0.05$
547 compared to basal values, $^{\#}p < 0.05$ compared to saline control group.
548

Figure 3. Morphological analysis of heart and lung samples dissected from anesthetized rats exposed to *M. l. lemniscatus* venom (0.1 and 0.3 mg/kg). Transversal sections of heart (A) and lung (B) samples from animals exposed to saline (A_1 and B_1) and venom 0.1mg/kg (A_2 and B_2) and 0.3 mg/kg (A_3 and B_3). It was not observed myonecrosis of cardiac muscle nor pulmonary hemorrhage and thrombosis formation. "b" - bronchial tube.; the bars in A_1 and B_1 represent 100 and 500 μm, respectively.

Figure 4. Vascular responses to *M. l. lemniscatus* venom (10 μg/ml) in rat isolated thoracic aorta preparations. Concentration-response curve to α1-adrenergic receptor agonist phenylephrine (PE) pre and post-venom incubation in strips with (E^+) (1) and without (E^-) (2) endothelium (A). Concentration-response curve to nitric oxide donor sodium nitroprusside (SNP) pre and post-venom incubation in strips E^- pre-contracted with PE (3 μM) (B). Concentration-response curve to muscarinic receptor agonist acetylcholine (ACh) pre and post-venom incubation in strips E^+ pre-contracted with PE (3 μM) (C). Relaxation induced by venom in strips E^+ pre-contracted with PE (3 μM) (D). The points (A–C) and columns (D) are mean ± SEM (n = 6); $*p < 0.001$ compared to pre-venom incubation curve (in C) and vehicle (in D).

Figure 5. Contractile force and atrial rate recorded from right and left rat isolated atria preparations. Amplitude of atrial contractile responses (A). Atrial rate (B). In A and B, the points are mean ± SEM (n = 5); $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$ compared to basal values in each group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ compared to control preparations (without venom). RA – right atrium, LA – left atrium, bpm – beats per minute.

Figure 6. Pharmacological strategies used in rat isolated thoracic aorta preparations to assess the signalling pathways potentially affected by *M. l. lemniscatus* venom to induce hypotension. (**1**) Venom seems to compete for endothelial muscarinic receptors (R_1) to induced vascular relaxation possibly via activating the NO synthesis pathway. (**2**) Venom did not interfere with the sGC activation by an exogenous NO donor (SNP). (**3**) Venom did not affect the vascular contractile mechanism activated by an α1-adrenergic receptor (R_2) agonist (PE). cGMP – cyclic guanosine monophosphate, CM – calmodulin, EC – endothelium cell, eNOS – endothelial nitric oxide synthase, GTP – guanosine triphosphate, MLCK – myosin light-chain kinase, MLCP – myosin light-chain phosphatase, NO – nitric oxide, PE – phenylephrine, R_1 – muscarinic receptor, R_2 – α1-adrenergic receptor, sGC – soluble guanylate cyclase, SNP – sodium nitroprusside, SR – sarcoplasmic reticulum, VSM – vascular smooth muscle.