Interleukin-1 receptor-induced PGE₂ production controls acetylcholine-mediated cardiac dysfunction and mortality during scorpion envenomation

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Scorpion envenomation is a leading cause of morbidity and mortality among accidents caused by venomous animals. Major clinical manifestations that precede death after scorpion envenomation include heart failure and pulmonary edema. Here, we demonstrate that cardiac dysfunction and fatal outcomes caused by lethal scorpion envenomation in mice are mediated by a neuro-immune interaction linking IL-1 receptor signaling, prostaglandin E₂, and acetylcholine release. IL-1R deficiency, the treatment with a high dose of dexamethasone or blockage of parasympathetic signaling using atropine or vagotomy, abolished heart failure and mortality of envenomed mice. Therefore, we propose the use of dexamethasone administration very early after envenomation, even before antiserum, to inhibit the production of inflammatory mediators and acetylcholine release, and to reduce the risk of death.
Severe scorpion envenomation induces pulmonary edema and cardiac dysfunction that can progress to fatal outcomes, especially in children and the elderly. *Tityus serrulatus* scorpion venom (TsV) is recognized by pattern-recognition receptors (PRRs), which induce the production of cytokines and lipid mediators, such as eicosanoids. TsV recognition by macrophages results in NLRP3-inflammasome activation and interleukin-1β (IL-1β) release, promoting lung edema and mortality. Inhibition of prostaglandin E2 (PGE2) production, amplification of IL-1β release, and abrogating both TsV-induced lung edema and mortality. Unfortunately, the development of new treatments has limitations, mainly due to a lack of information about the mechanisms involved in the etiology of clinical manifestations. This includes cardiac dysfunction, which is considered one of the main pathophysiological events associated with the high index of scorpion envenomation-induced mortality.

Currently, it is known that toxins present in TsV activate ion channels that induce a neuroexcitatory syndrome known as a neurotransmitter storm, an excessive response associated with hyperactivation of the autonomic nervous system, which leads to TsV-induced cardiogenic shock and other systemic manifestations. The autonomic nervous system is divided into parasympathetic and sympathetic systems that perform opposite functions in the maintenance of homeostasis through the release of acetylcholine (ACh) and catecholamines. The excessive release of ACh is responsible for bradycardia, cardiac arrhythmias, elevated nasal, salivary, lacrimal, pancreatic, and bronchial secretions, along with piloerection and muscle spasms, while exaggerated catecholamine production mediates cardiac arrhythmias, tachycardia, arterial hypertension, shock, hyperglycemia, and leukocytosis. Depending on the intensity of these manifestations, envenomed mammals can progress to severe arterial hypotension and, subsequently, death. Although the contribution of these neurotransmitters to cardiogenic shock and death has been recognized during severe scorpion envenomation, the molecular mechanisms underlying their transmission remain unclear. Recent progress in interdisciplinary research has described the relevance of (i) the immune system in the physiological control of the cardiovascular system and (ii) neural signals in the regulation of cardiovascular functions, as well as in the heart immune response. With these studies in mind, we hypothesized that TsV-induced cardiac dysfunction and subsequent mortality occur through neuroimmune interactions via a specific brain–heart neural circuit. By combining genetic, surgical, and pharmacological approaches, we set out to investigate the neurotransmitter storm mechanistically by exploring the hierarchy of neural and molecular events connecting autonomic with the heart innate immune response.

Here, we show that scorpion envenomation induces autonomic hyperactivation and heart inflammation in mice. Cardiac fibroblasts respond to TsV and produce PGE2 and IL-1β in vitro. Furthermore, IL-1R signaling is required for sustained PGE2 production, amplification of IL-1β release, cardiac dysfunction, and mortality in vivo. Indeed, PGE2 signaling via EP2/4 receptors mediates excessive ACh release, which in turn causes the heart failure of envenomed mice. Of importance, we introduce a novel pathway that is targeted by dexamethasone, but not by anti-scorpion venom serum. Taken together, our study indicates that suppression of the inflammatory response can be an effective supportive therapy to prevent cardiac dysfunction and mortality caused by scorpion envenomation.

**Results**

TsV provokes autonomic dysfunction and heart inflammation. Lethally envenomed C57BL/6 (WT) mice (TsV, 180 µg kg⁻¹) exhibited sweating, ocular secretion, nasal secretion, lethargy, and convulsion, referred to here as systemic autonomic manifestations in order to create a clinical score and evaluate the severity of envenomation (Fig. 1a). We also detected hyperglycemia (Fig. 1b), a clinical sign that is also observed in scorpion-envenomed patients. Next, to investigate the impact of venom administration on the autonomic systems, we measured the amount of sympathetic and parasympathetic neurotransmitters in the blood, specifically catecholamines (adrenaline and noradrenaline) and ACh, respectively (Fig. 1c–e). We detected a dramatic increase in the concentration of adrenaline and ACh compared to PBS-inoculated mice (control) (Fig. 1c, e). Surprisingly, although studies have suggested that these changes are caused by excessive activation of both the sympathetic and parasympathetic systems, no studies have identified the mechanisms involved in these scorpion-induced autonomic disturbances, particularly those involved with the cardiovascular system. In lethally envenomed mice, within 60 min, we observed cardiac disorders with diminished mean arterial pressure and heart rates (Fig. 1f, g), which rapidly culminated in death in 50–100% of cases. We also detected peripheral blood leukocytosis (a hematologic manifestation that is also provoked by sympathetic hyperactivation), mainly due to neutrophilia and also due to a reduction in the number of mononuclear cells (Fig. 1h, j).

Given that one of the leading causes of death by scorpion envenomation is cardiovascular dysfunction, we sought to determine the effect of scorpion venom on the induction of an inflammatory response in the heart. Just as we observed in the blood, flow-cytometry analysis of leukocytes in the heart demonstrated an increase in the neutrophil influx and a decrease in macrophages (Fig. 1k, l), which were accompanied by the upregulation of genes involved in innate immune responses and eicosanoid metabolism (Fig. 1m and Supplementary Table 1). Moreover, TsV increased NLRP3, Asc, and Casp1/11–dependent IL-1β release (Fig. 1n), as well as PGE2 production in the heart (Fig. 1o) and PGE2 in the serum (Fig. 1p). These data confirm that lethally envenomed mice present clinical autonomic manifestations similar to those observed in humans, and that TsV induces a pro-inflammatory response in the heart, an organ richly innervated by both branches of the autonomic system.

**Cardiac fibroblasts mediate the inflammatory response to TsV.**

Given the pro-inflammatory profile observed in the hearts of TsV-envenomed animals, next, we wished to determine which cardiac cell(s) contribute to the inflammatory process. Upon stimulating primary cultures of either cardiomyocytes (CMs) or cardiac fibroblasts (CFs) with TsV for 24 h, we found that only CMs released IL-1β (Fig. 2a), while both cell types produced PGE2 (Fig. 2b). Moreover, TsV-stimulated CFs exhibited higher expression of inflammatory genes and increased cell surface expression of CD14 and TLR4, but not TLR2 (Supplementary Table 2 and Fig. 2c, respectively). It is known that at least two signals are required to activate the NLRP3 inflammasome and to boost IL-1β maturation and release by macrophages. Following TsV exposure, these signals include PRR signaling, potassium (K⁺) efflux, PGE2 release, production of 3′,5′-cyclic adenosine monophosphate (cAMP), protein kinase A (PKA) activation, and enhanced nuclear factor-κB (NF-κB) activity. Interestingly, TsV-stimulated CFs also require K⁺ efflux and PGE2-EP2/cAMP/PKA signaling to produce IL-1β, which was impaired under culture conditions with elevated concentrations.
of extracellular K\(^+\) (but not NaCl) (Fig. 2d), an antagonist of prostaglandin receptors (compound AH6809) (Fig. 2e), or a PKA inhibitor (compound H89) (Fig. 2g). Moreover, in TsV-stimulated CFs, cAMP levels increased after the addition of exogenous PGE\(_2\) (Fig. 2f). We also observed the upregulated expression of total and phosphorylated forms of PKA in heart lysates of envenomed mice (Fig. 2h). These data suggest that TsV causes CFs to release IL-1\(\beta\) via NLRP3-inflammasome activation, which can be enhanced by the PGE\(_2\)/cAMP/PKA/NF-κB pathway, demonstrating that, aside from systemic inflammation, TsV induces heart inflammation by stimulating CFs to produce IL-1\(\beta\) and PGE\(_2\).
Impact of the cardiac inflammation induced by envenomation. To determine the impact of local and systemic inflammation on heart function, we used mice deficient for the IL-1 receptor (IL-1R) or suppressed the inflammatory response with a high dose of corticosteroid. Deficiency of IL-1R (Il1r1−/−) or treatment of WT-envenomed animals with dexamethasone (DEX) abolished the previously observed alterations in mean arterial pressure and heart rate (Fig. 3a, b). The echocardiographic analysis showed that only untreated-WT-envenomed mice presented cardiac dysfunction, as estimated by reductions in the stroke volume (SV; the volume of blood pumped out of the left ventricle of the heart during each systolic cardiac contraction), ejection fraction (EF; measurement, expressed as a percentage, of how much blood the left ventricle pumps out with each contraction) and cardiac output (CO; the volume the heart pumps through the systemic circulation over a period measured in liters per minute) (Fig. 3c, d). Interestingly, WT-envenomed animals treated with DEX or Il1r1−/−-envenomed mice were resistant to fatal outcomes (Fig. 3e, j, respectively) and exhibited reduced clinical scores (Fig. 3f, k, respectively). Resistance to TsV envenomation correlated with a significant reduction in the concentration of IL-1β in the heart (Fig. 3g, l, respectively), as well as in the concentration of PGE2 in the heart (Fig. 3h, m, respectively) and the serum (Fig. 3i, n, respectively). Collectively, these data indicate the pivotal role of inflammation in cardiac dysfunction and mortality during severe scorpion envenomation.

The above-mentioned results revealed the crucial role of inflammation in heart failure induced by scorpion venom. Therefore, we hypothesized that rapid inhibition of venom-induced inflammation might be a useful therapy to treat scorpion envenomation, in addition to antiserum administration. To explore the potential use of an anti-inflammatory as a supportive therapy for scorpion envenomation, a proof-of-concept experiment was conducted. Lethally envenomed animals showed a decrease in critical cardiac parameters, such as ejection fraction, stroke volume, and cardiac output (Fig. 4a), and, as expected, DEX treatment 30 min after TsV inoculation prevents these venom-induced cardiac dysfunctions (Fig. 4b). Interestingly, the
administration of specific anti-scorpion venom serum modified the drop in ejection fraction, but not the stroke volume or the cardiac output alterations induced by the venom (Fig. 4c). However, DEX treatment 15 min after the venom inoculation and 15 min before the subsequent administration of anti-scorpion venom serum (given 30 min after TsV) prevented heart failure (Fig. 4d). The echocardiographic images reflect the effect of the treatments on mouse heart function (Fig. 4e), whereby treatment with DEX preserved all the features and characteristics of a standard cardiac chamber. Taken together, these results suggest that DEX has the potential to be used as supportive therapy to prevent the occurrence of potentially lethal cardiac changes.

**TsV induces heart failure via PGE2-dependent ACh release.** As revealed before in clinical studies (and reproduced experimentally in this study; Fig. 1a–e), scorpion envenomation induces autonomic hyperactivation. In our research, we also found increased concentrations of catecholamines (adrenaline and ACh) in the plasma of lethally envenomed WT mice (Fig. 1c, e); however, the molecular mechanisms through which these neurotransmitters regulate cardiac dysfunction, clinical score, and mortality are still unknown. We verified that antagonizing betadrenoceptors with propranolol improved neither animal survival nor the clinical score or high cardiac IL-1β production (Fig. 5a–c). Interestingly, in contrast to treatment with propranolol, the administration of atropine, a nonselective muscarinic antagonist, rescued the mice from mortality (Fig. 5d) and reduced the clinical score (Fig. 5e) without interfering with IL-1β production in the heart (Fig. 5f). Additionally, atropine administration prevented all the observed and evaluated cardiac alterations, reflected by preserved EF, SV, and CO, as observed via echocardiographic analysis (Fig. 5g, h).

The vagus nerve is the main parasympathetic nerve and the most important neural source of peripheral ACh, which exerts its cardiac functions mainly by muscarinic receptor activation. Given that muscarinic receptors participate in scorpion venom-
induced autonomic dysfunctions, we next investigated the involvement of parasympathetic signaling after TsV envenomation. It has already been described that right cervical vagotomy decreases peripheral ACh concentration 15; thus, we took advantage of this knowledge to define the relationship between the vagus nerve and TsV envenomation. Mice were vagotomized or sham-operated 5 days before lethal TsV envenomation. We observed that right cervical vagotomy protected the mice from envenomation, preserving the cardiac functions (EF and CO), as demonstrated by echocardiographic analysis (Fig. 5g, h).

**Fig. 4 Efficacy of therapy with dexamethasone and/or antiscorpionic serum.** Ejection fraction (EF), stroke volume (SV), and cardiac output (CO) of C57Bl/6 mice were submitted to echocardiography before (basal) and 45 min after inoculation with the venom. a TsV only; b TsV plus DEX (5 mg kg$^{-1}$ i.p.)/15 min after the venom; c TsV plus antiscorpionic serum (30 µl/1 mg/ml/animal)/30 min after the venom; or d TsV plus DEX (5 mg kg$^{-1}$ i.p.)/15 min after the venom followed by antiscorpionic serum (30 µl/1 mg/ml/animal)/15 min after DEX ($n$ = 5–6, one experiment). e Representative M-mode image of echocardiographs obtained from the above-mentioned groups. Data are expressed as means ± SEM. Paired t test was applied to evaluate statistical significance. Differences were considered significant if $P < 0.05.$
Together, these results suggest that both pharmacological (atropine) and surgical (vagotomy) approaches can block the inflammation-regulated parasympathetic signaling that controls severe cardiac dysfunction.

Since IL-1R deficiency and DEX treatment prevent PGE2 production, cardiac dysfunction, and mortality, and with the knowledge that PGE2 stimulates neuronal ACh release16,17, we next investigated its association with ACh release in scorpion envenomation. Interestingly, the absence of IL-1R-signaling and treatment with DEX or indomethacin (Indo, a COX1/2 inhibitor) both abrogated TsV-induced systemic ACh release (Fig. 5i, j) and PGE2 production in the lungs (Fig. 5k). Because indomethacin...
treatment of envenomed WT mice inhibited ACh release (Fig. 5i) and Il1r1−/− animals produced lower concentrations of PGE2 after TsV envenomation (Zoccol et al.2) compared to WT mice (Figs. 3m, n and 5k), we hypothesized that IL-1R-induced PGE2 production controls systemic ACh release during scorpion envenomation. In support of this hypothesis, we discovered that Il1r1−/− mice only exhibited increased systemic ACh when TsV was inoculated at the same time as exogenous PGE2 (Fig. 5i). Next, we confirmed the functional role of PGE2 in TsV-induced ACh release pharmacologically by antagonizing EP2/EP4 receptors (AH6809 and AH23848, respectively) in Il1r1−/−-envenomed mice (Fig. 5l) or by selectively inhibiting microsomal PGE synthase-1 (mPGES-1) (CAY10526) in envenomed WT animals (Fig. 5m). To investigate whether PGE2 can inhibit the AChE activity, we measured AChE activity in the serum. We observed no differences in the activity of serum AChE in either WT or Il1r1−/− envenomed mice with or without CAY10526 or PGE2 treatment (Fig. 5n, o). Altogether, our results demonstrate that cardiac dysfunction and mortality induced by TsV depend on a neuroimmune pathway involving IL-1R-induced PGE2 production, which in turn stimulates ACh release via EP2/EP4 signaling. The mechanism is summarized in Fig. 6.

**Discussion**

The hyperactivation of the autonomic system and excessive inflammation during scorpion envenomation have been previously documented14; however, the mechanisms involved in venom-induced cardiovascular dysfunction and mortality through manipulation of the autonomic system have never been investigated, particularly from a neuroimmune perspective. In the present study, we established, for the first time, a direct relationship between the inflammatory process, neuronal activation, cardiac dysfunction, and mortality induced by scorpion venom. Also, we demonstrated that although both the parasympathetic/vagal and sympathetic systems are excessively activated after scorpion venom administration, only the blockade of the first prevented the cardiovascular alterations and mortality typically observed in envenomed mice.

Inoculation of TsV induces systemic changes in WT mice, similar to those observed in humans14. However, the impact of scorpion venom on heart inflammation and its connection with the release of neuro-mediators release has never been deeply explored. In this investigation, as previously described in macrophages and lungs2,4,18, we showed that CFs of WT mice recognized the venom and increased the expression of inflammatory genes coding for COX-2 and EP2 receptor, as well CD14 and TLR4 PPR, in addition to the release of PGE2 and IL-1β, through mechanisms regulated by cAMP and PKA. We also confirmed that the NLRP3-inflammasome platform is responsible for IL-1β production in the heart and CFs via a mechanism similar to that first described in macrophages4, Previous studies have shown that CFs express PRRs that recognize pathogen-associated molecular patterns (PAMPs), damage-associated molecular patterns, and venom-associated molecular patterns (VAMPs)2,19–21, and the increase in NLRP3 expression in CFs has been implicated in the size of the infarcted area during ischemia/reperfusion22, and is crucial for cardiac dysfunction during sepsis23. Interestingly, the role of IL-1β in cardiac dysfunction has already been demonstrated in previous studies20–24–27, given that this cytokine can desensitize cardiomyocytes after adrenergic stimulation and induce reversible contractile dysfunction in the cardiac muscle along with arrhythmias24. However, in addition to the mediators produced by the heart cells, we also have to consider the contribution of PGE2 and IL-1β produced both systemically and by the lungs to cardiac dysfunction. We have previously reported that after scorpion envenomation, the main source of PGE2 are resident macrophages in the lungs, but the contribution of other peripheral mononuclear cells cannot be discarded. However, further studies are necessary to better assess this important aspect4. Curiously, and contrary to our previous observation in macrophages4, TsV did not increase cAMP production by cardiac fibroblasts in vitro, unlike the high amount of exogenous PGE2 in vitro (10 μM). Since cardiac fibroblasts are naturally low PGE2 (5 nM, Fig. 2a) and high cAMP (Fig. 2h) producers, 28 a high concentration of PGE2 might be necessary to boost additional cAMP production by heart cells in vivo, and this could be a mechanism to prevent excessive activation of cardiac fibroblasts and interstitial fibrosis28. On the other hand, high concentrations of PGE2 are apparently necessary to promote ACh release, as observed in Il1r1−/− TsV- and PGE2-inoculated mice (35 μM per animal, Fig. 5f). In WT TsV-envenomed mice, lungs provided a higher concentration of PGE2.

Challenging IL-1R-deficient animals (Il1r1−/−) or DEX-treated WT mice with a lethal dose of TsV confirmed the involvement of
the inflammatory mediators PGE2 and IL-1β in the systemic manifestation, cardiac dysfunction, and mortality (Fig. 3). Because scorpion venom induces the release of catecholamines and ACh, and knowing that the autonomic system controls the cardiovascular, gastrointestinal, and urogenital systems and also regulates the immune responses, we next investigated the contribution of these neurotransmitters to cardiac dysfunctions, clinical score, and mortality. Pharmacological approaches and vagotomy confirmed the relevance of ACh for systemic and cardiac manifestations of severe scorpion envenomation. Additionally, in this study, we revealed that the sequential activation of parasympathetic (vagal) signaling and muscarinic receptors, which are the final steps in the IL-1β/IL-1R/PGE2 inflammatory axis in the heart, promotes severe TsV-induced cardiovascular dysfunction. Different studies have demonstrated interactions between PGE2 and ACh synthesis. For example, EP4 activation in a leukemic T-cell line increases ACh production. Also, it is known that the stimulation of the vagus nerve promotes the synthesis of ACh through a mechanism involving T cells, which act as a non-neural source of this neurotransmitter. Similar to our results, a previous study has demonstrated the relationship between the release of this ACh and mPGES-1-dependent PGE2 production in neuroimmune vagal activity. However, findings in the literature are controversial. Some studies confirm that electrical stimulation of the vagus nerve controls exacerbated inflammatory responses, such as in rheumatoid arthritis and Crohn’s disease. Other neuroimmune pathways and bioelectronic approaches involving autonomic signaling have been studied in inflammatory conditions. In contrast, other studies have demonstrated that autonomic dysfunctions are involved in the pathophysiology of different immunosuppressed and cardiovascular conditions mediated by neuroimmune circuits. For example, hyperactivation of the parasympathetic and sympathetic nervous systems after acute central nervous system injury, like stroke and spinal cord injury, leads to an immunosuppressive state favoring infectious complications, such as pneumonia.

Also, chronic psychosocial stress activates sympathetic activity, promoting the differentiation of hematopoietic stem cells and acceleration of the formation of plaques, such as those found in atherosclerosis. Finally, it is crucial to mention that, although ACh has been initially described as an anti-inflammatory neurotransmitter, current data have suggested contradictory conclusions. The data described in this paper are fundamental for the comprehension of human scorpion accidents because they reveal for the first time a neuroimmune response influencing the parasympathetic pathway after scorpion envenomation, which is responsible for cardiac failure. In agreement with our results, a recent study described a neuroimmune cholinergic pathway mediated by sympathetic, but not parasympathetic, activation responsible for immune activation, and hypertension development. It is plausible that hyperactivation of the cholinergic pathways mediates excessive ACh (direct) or catecholamine (indirect) release after inflammatory stimuli, generating opposite hemodynamic effects and therefore promoting severe hypotensive and hypertensive responses, respectively. Studying interactions between the nervous and immune systems has introduced new concepts for organism functioning and has suggested neuroimmune approaches as attractive alternative treatments. In this study, surgical (vagotomy) and pharmacological (atropine) blockage of parasympathetic signals improved TsV-induced cardiac dysfunctions and mortality. Curiously, previous studies have demonstrated that blocking neural signaling protects against infectious and autoimmune diseases. Our pharmacological findings highlight the possibility that muscarinic antagonists may...
have a positive impact on mortality and several cardiovascular dysfunctions after venom administration. However, although supportive therapy with atropine has already been indicated for severe bradycardia and hypotension, it must be used with caution¹ to avoid unwanted side effects such as dry mouth, nausea, and death. On the other hand, muscarinic antagonists and agonists involved in the vagal activation or cardiac cells could be used selectively to reduce side effects⁴⁶. Selective muscarinic-2 antagonist drugs could be candidates for treating scorpion envenomation since this receptor sub-type exerts parasympathetic activities in the heart rate and atrioventricular nodal conduction. However, although we reported here that the blockade of parasympathetic signals by vagotomy or atropine administration improves the survival of envenomed mice, the precise immuneun mechanisms by which PGE₂-EP2/EP4 signaling induces ACh release remains to be determined.

Typically, the treatment of scorpion envenomation involves only the administration of antivenom serum, and in some hospitals, a non-immunosuppressive corticosteroid dose is given at the same time as the serum to prevent serum sickness⁸. However, although administration of antivenom serum is recommended, a treatment to neutralize neurotoxins presents in scorpion venom⁹ it is not easily accessible in several clinical ambulatories. Moreover, the antiserum administration to envenomed patients does not control the massive release of inflammatory mediators. Here, as a proof-of-concept, we demonstrated that despite the beneficial impact of the antiserum administration, it addresses only the decrease of ejection fraction (Fig. 4). In contrast, the administration of a high dose of the anti-inflammatory DEX, which inhibits PGE₂ and IL-1β (Fig. 3), 30 min after challenging the mice with a lethal dose of scorpion venom, but before administration of the antiserum, prevents cardiac dysfunction. Interestingly, the administration of DEX before the antiserum restores the cardiac function to the same level as non-envenomed animals. DEX is the most effective anti-inflammatory drug used to inhibit PGE₂ and IL-1β⁴⁵, is used worldwide, has a low cost, and is easily found in the most remote regions of the globe.

Therefore, in scorpionism, our findings encourage the early administration of a high dose of DEX to block the production of inflammatory mediators, and consequently, the release of ACh and heart failure, especially in situations in which delayed antiserum treatment increases the risk of death. Translational research beyond murine models is needed to explore the potential strategy of neuroimmunomodulation in the treatment of scorpion envenomation.

**Methods**

*Tityus serrulatus* venom (TsV). Lyophilized venom obtained from yellow scorpions (*Tityus serrulatus*) used in this study was provided by the Butantan Institute, São Paulo, SP, Brazil. Briefly, the venom was extracted by electric stimulation followed by freeze-drying, and was stored at −20 °C. For all experiments, TsV was diluted in sterile PBS (phosphate-buffered saline) and filtered in a 0.22-µm sterilizing membrane (Millipore, USA). Next, LPS detection test was performed (LAL; QCL-1000, Bio Whittaker, Cambrex Company, USA) according to the manufacturer’s instructions. In all TsV samples, LPS was not detected.

**Animals.** For the primary culture of cardiac fibroblasts (CFS) and cardiomyocytes (CMs), newborn C57Bl/6 mice (1-2 days) obtained from the animal facilities of the Faculdade de Medicina de Ribeirão Preto, University of São Paulo (FMRP-USP), were used. For in vivo studies, adult male C57Bl/6 (6-8 weeks) and IIt1 mice animals matched by age and weight in all procedures were obtained from the animal facilities of FMRP-USP. Mice were maintained in a light/dark cycle with free access to food and water, room temperature (25 °C), in 40–60% of humidity. Maintenance and the experiments with mice were conducted in accordance with the Ethical Principles in Animal Research adopted by the National Council for the Control of Animal Experimentation (CONCEA), and were approved by the Animal Care and Use Committee of the Faculdade de Ciências Farmacêuticas de Ribeirão Preto (CEUA-FCFRP) at the Universidade de São Paulo (FCFRP-USP), Ribeirão Preto, São Paulo, Brazil (Process no. 161.1081.60.5) and by the Animal Committee of FMERP-USP (002/2018-1).

**Primary cultures of cardiac cells and in vitro pharmacological treatments.** Two hundred newborn mice were used for a yield of 10 plates of 24-well cells. The hearts from neonatal C57Bl/6 mice (1–2 days) were withdrawn within the laminar flow chamber and digested afterward with collagenase type II (Worthington Biochemical Group, Lakewood, USA) digestion buffer. After five cycles of digestion (digestion-centrifugation-exchange of digestion buffer), the CMs and CFS were rinsed with PBS, 2 mg kg⁻¹ of TsV in a glucose-enriched medium (25 mM) with newborn calf serum (NBCS: 10%) and the antibiotics penicillin and streptomycin (100 U/mL), and then kept in incubators for 24 h at 37 °C with 5% CO₂. For all experiments, CMs (4 x 10⁶ cells/well) or CFS (8 x 10⁶ cells/well) were incubated with medium or with 50 μg ml⁻¹ of TsV for 24 h. The electrophysiological and contractile responses were performed in different sets of experiments: prostaglandin E₂ (PGE₂) ( Cayman Chemical Company, USA), 10 μM, 10 min before TsV stimulation; prostaglandin E₂ receptor 2 (EP2) antagonist AH6809 (Cayman Chemical Company, USA), 1 μM, 30 min before TsV stimulation; KCl/NaCl (Sigma-Alrich/Merck, USA), 0.1 mmol l⁻¹, 15 min before TsV for 24 h; and protein kinase A (PKA) inhibitor, H89 compound (Sigma-Alrich/Merck, USA), 25 μM, 2 h before TsV stimulation. In all experiments, except for CAMP measurement (5 min), the supernatants were collected for IL-1β and PGE₂ quantification 24 h after TsV stimulation. The adhered cells were used for qRT-PCR. The protocol was adapted from Zoccal et al.¹¹.

**In vivo experiments and drug treatments.** In all in vivo experiments and under all conditions, C57Bl/6 and IIt1 adult mice (22–25 g) were weighed and then anesthetized intraperitoneally (i.p.) with 100 μg kg⁻¹ of TsV, 180 μg kg⁻¹ of atropine and 1% alcohol in PBS, 2 mg kg⁻¹ of mifepristone (0.61-mm external diameter and 0.28-mm internal, Clay-Adams) were inserted into the left carotid artery to record blood pressure. The arterial catheter was connected to a pressure transducer (DPT-100, Deltran®, Utah Medical Products Inc, USA), and the blood pressure was continuously sampled (4 kHz) using a computer equipped with an analog-digit al interface (PowerLab/SP, ADInstruments, Colorado Springs, CO, USA). The baseline blood pressure was measured for 30 min before TsV injection, and recorded for 240 min after TsV inoculation. The hearts and lungs were removed and perfused before being digested in sterile water for the quantification of IL-1β and PGE₂ (Supplementary Fig. 1a, b). Based on the time course of the production of IL-1β and PGE₂ after TsV, 60 min (the peaks of the two mediators) was chosen to perform all of the following experiments in vivo and for obtaining hearts, lungs, and blood, except for animals treated with indomethacin (Indo) or dexamethasone (DEX) for ACh quantification (Fig. 2f), for which mice were also euthanized 75 and 120 min after the venom inoculation. Hearts, and in some experiments lungs, were employed for RT-qPCR, western blotting, and histological analysis. For the measurement of blood pressure and heart rate, C57Bl/6 and IIt1 mice were anesthetized with a mixture of 2% isoflurane and 100 O₂, and kept at a controlled temperature and heart rate. Doppler echocardiographic examination was performed using the Vevo 2100 System echocardiograph (Visual Sonics, Toronto, ON M4N 3N1, Canada) with a 30 MHz probe. The animals were positioned to obtain the long axis and to record images. Using two-dimensional visualization of the left ventricle in the long axis, the end-diastolic and end-systolic images were obtained from the M-mode image, the following parameters were obtained (in mm): diastolic interventricular septum thickness, diastolic left ventricular internal diameter, diastolic left ventricular free wall, systolic interventricular septum thickness, left ventricular internal diameter in systole, and left ventricular free wall thickness in systole. The shortening fraction (%) was calculated from these values, and the contraction fraction, stroke volume, and cardiac output were calculated by the Teichholz method. In all pharmacological treatments, 300 μl of the specific dilutor of each drug (vehicle) or PBS was injected in animals with or without TsV inoculation. The following pharmacological treatments were administered in C57Bl/6 or IIt1 mice: Dexamethasone (Aché Pharm) or Atropine (Pharmaceutical Laboratory, USA; were obtained in 0.9% NaCl, 1.15 mmol l⁻¹ or 15 mmol l⁻¹ or 15 min after TsV inoculation; mPGES-1 specific inhibitor CAY10526 (Cayman Chemical Company, USA), 1% alcohol in PBS, 5 μg kg⁻¹ i.p., 24 h and 60 min before TsV inoculation; PGE₂ (Cayman Chemical Company, USA), PPS + 0.01% of ethanol, 1 μg kg⁻¹ i.p., Twenty-four hours and 15 min before TsV inoculation; propranolol (Sigma-Aldrich/Merck, USA), 300 μl in PBS 4 mg kg⁻¹ i.p, 60 min before TsV inoculation; atropine (Sigma-Aldrich/Merck, USA) 300 μl in PBS, 2 mg kg⁻¹ i.p. Sixty minutes before TsV inoculation; indomethacin...
**Flow cytometry.** Heart cell suspensions were obtained after tissue digestion at 37 °C for 60 min in 1 ml of heart digestion buffer (RPMI 1640, Liberase LT, Roche, Basel, Switzerland, and DNase 0.5 mg ml⁻¹, Sigma-Aldrich, St. Louis, MO, USA). The tissue fragments were passed through a cell strainer with 100-μm pore size (BD Biosciences, Franklin Lakes, New Jersey, USA). Next, the red blood cells were lysed, and the remaining cells were washed in PBS, centrifuged, and resuspended in RPMI 1640 containing 5% FBS. Suspensions of 1 × 10⁶ cells from heart tissue were used for further analysis. The following antibodies were used: CD11b (clone: M1/ 70; BD Biosciences), Ly6C (clone: RB6-8C5; BioXcell, clone: M3/145.2); F4/80 (clone: BM8); CD31 (clone: EPR17259); PDGFRα (clone: APA5); CD4 (clone: rmC5-3); TLR4 (clone: MTS510); and TLR2 (clone: 6C2). The antibodies used for flow cytometry were purchased from eBioscience (San Diego, CA), BD Biosciences (Franklin Lakes, New Jersey, USA), or Abcam (Cambridge, UK). Data acquisition was performed using a BD LSORTess flow cytometer and FACSDiva software (BD Biosciences, Franklin Lakes, New Jersey, USA). In total, 1,000,000 events were acquired for samples. Data were plotted and analyzed using FlowJo software v.10.0.7 (Tree Star, Inc, Ashland, OR, USA). Gating strategies are shown in Supplementary Fig. 2.

**Quantitative PCR with reverse transcription.** Heart lysates or cardiac fibroblasts were used for RT-qPCR. RNA was extracted using RNA-extractions kits according to the manufacturer’s instructions (PurElutin, Ambion, USA), and the quantification was determined by fluorometric assays (Qbit, Invitrogen, CA, USA). Complementary DNA (cDNA) was synthesized from 1 μg of total RNA using Taqman primers for 10 min at 95 °C followed by 40 cycles of 15 s at 60 °C and 60 °C for 20 s. The 2⁻ΔΔCt method was used for all samples for analysis. For the heatmap, the n-fold difference relative to control (all gene expressions was normalized to Gapdh) was used to plot the results using the Heatmapper platform.

**Western blotting.** Hearts from C57Bl/6 envenomed mice were removed and digested in RIPA buffer (Sigma-Aldrich/Merck, Darmstadt, Germany). Next, protein quantification of the supernatant was performed using Lowry reagents (DC™ Protein Assay, Bio-Rad, USA). The supernatants were suspended with LDS sample buffer (4×) (Life Technologies, USA), boiled, resolved by SDS-polyacrylamide gel electrophoresis (10–15% gel), and transferred onto a 0.22-μm nitrocellulose membrane (GE HealthCare, USA). The membranes were blocked with Tris-buffered saline containing 0.1% Tween 20 and 5% nonfat dry milk. The monoclonal antibodies to PKA-γ clone [EP2647Y] (Abcam, USA) and PKA (phospho T197) clone [EP2606Y] (Abcam, USA) were diluted in blocking buffer for incubation. Antibody detection was done using Enhanced Chemiluminescence Reagent (GE Healthcare, USA).

**Measurement of intracellular cAMP.** For intracellular cAMP measurement, 8 × 10⁶ cells were cultured with 0.1 M HCl, 10 µM PGE₂, 0.05 mg g⁻¹ of ACh or with or without PGE₂ (10 µM) for 3 min before stimulation with TxA₂ (50 µg ml⁻¹) for 5 min. Cell culture supernatants were aspirated, and the cells were lysed by incubation for 10 min with 0.1 M HCl, followed by disruption using a sterile syringe. Intracellular cAMP was quantified by ELISA using an acetylation protocol according to the manufacturer’s instructions (Enzo Life Sciences, NY, USA).

**Quantification of IL-1β, PGE₂, acetylcholine (ACh), and catecholamines.** IL-1β in the supernatant of heart homogenates or cell culture supernatants were quantified using an IL-1β ELISA kit (BD Biosciences, USA). To obtain plasma or serum, animals were anesthetized with ketamine plus xylazine, and blood was collected by cardiac puncture with heparinized or not syringes. For plasma or serum separation, the blood was centrifuged for 500 g for 10 min at 4 °C. After separation, plasma or serum was immediately frozen in liquid nitrogen until the quantification was performed. For PGE₂ quantification, the supernatant of heart homogenate or serum was previously treated in Sep-Pak C₁₅ cartridges (Waters Corp.), and the measurement was performed using the EIA assay (Enzo Life Sciences). ACh quantification was performed in the plasma using a colorimetric assay according to the manufacturer’s instruction sham animals (Cholinesterase/Acetylcholine assay kit, Abcam, USA). Briefly, free choline was measured, and, using the same sample, the acetylcholinesterase enzyme was added to the sample, and the total choline was measured again. The total choline was subtracted from free choline, resulting in the amount of ACh in plasma samples. Plasma catecholamines (adrenaline and noradrenaline) were measured using HPLC (JLC/2AT Shimadzu Prominence, Japan) coupled to an electrochemical detector (Decade Life-Antec Scientific, Netherlands) with a 5-µm Spherisorb ODS-2 reversed-phase column (Sigma-Aldrich, USA). Acetylcholinesterase activity was determined in plasma according to the manufacturer’s instructions (Acetylcholinesterase assay kit, Abcam, USA).

**Vagotomy and sham surgery.** A unilateral cervical vagotomy technique was performed. Briefly, the animals were submitted to analgesia with ketamine (0.05 µg g⁻¹) and xylazine (0.02 µg g⁻¹). A median cervical ventral incision was made to expose the right vagal trunk, which was then separated from the carotid artery and sectioned. In sham animals, the surgical intervention was similar to the denervated group, but without sectioning of the vague nerve. Five days after the procedure, the animals were inoculated with a lethal dose of TsV and, after 60 min, the humanitarian endpoint, mice were euthanized by cervical dislocation, preceded by anesthesia with ketamine and xylazine.

**Statistical analysis.** We performed Student’s t test for two-group comparison, and one-way analysis of variance (ANOVA) followed by Bonferroni’s multicomparison test for comparison of multiple groups. For survival analysis, the log-rank test was used for the determination of statistical significance. Specifically for echocardiogram analysis, paired t test was performed. In all tests, differences with P < 0.05 were considered statistically significant.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

All data supporting the results reported here are available upon reasonable request to the corresponding author. Source data are provided with this paper.

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