ELSEVIER

European Journal of Pharmacology 363 (1998) 139–146

ejp

Activation by *Phoneutria nigriventer* spider venom of autonomic nerve fibers in the isolated rat heart

Soraia K.P. Costa [a,*], Stephen Hyslop [a], Luciana P. Nathan [a], Angelina Zanesco [a], Susan D. Brain [b], Gilberto de Nucci [c], Edson Antunes [a]

[a] *Department of Pharmacology, Faculty of Medical Sciences, UNICAMP, PO Box 6111, 13083-970, Campinas, SP, Brazil*
[b] *Pharmacology Group and Vascular Biology Research Centre, Biomedical Sciences Division, King's College, Manresa Road, London, SW3 6LX, UK*
[c] *Department of Pharmacology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, SP, Brazil*

Received 12 October 1998; accepted 20 October 1998

Abstract

In the isolated rat heart, *Phoneutria nigriventer* spider venom (10–100 μg) produced a dose-dependent and reversible rise in left ventricular developed pressure. A low dose (10 μg) of venom induced a short-lasting, positive inotropic effect ($P < 0.05$) with no change in heart rate or coronary flow. At a dose of 50 μg, the venom caused significant positive inotropic and chronotropic responses associated with occasional ventricular arrhythmia, whereas coronary flow was not significantly affected within 10 min after venom administration. The highest dose of venom (100 μg) caused bradycardia, transient cardiac arrest, rhythm disturbances and an increase in end diastolic pressure followed by a reduction in coronary flow. Hearts treated with the non-selective β-adrenoceptor antagonist propranolol (3 μM) and the selective β_1-adrenoceptor antagonist CGP-20712A (10 μM) were protected against all the cardiac actions of the venom. The selective β_2-adrenoceptor antagonist butoxamine (10 μM) slightly reduced the cardiac response to 50 μg, but not to 100 μg of venom. Butoxamine also prevented the reduction in coronary flow induced by 100 μg of venom. Hearts from reserpine-treated rats (5 mg kg^{-1} day^{-1}, i.p., for 2 days) showed a marked decrease in all venom (≤ 100 μg)-induced cardiac responses. The muscarinic receptor antagonist atropine (1 μM) slightly potentiated the response to 50 μg of venom but had little or no effect on the responses to 100 μg of venom. The cardiac responses to venom (50–100 μg) were unaltered in hearts from rats treated with 8-methyl *N*-vanillyl-6-nonenamide (capsaicin; 50 mg/kg, s.c.). These findings indicate that *P. nigriventer* venom releases norepinephrine from cardiac sympathetic nerve endings and this may explain the observed increase in contractile force and heart rate.

Keywords: Spider venom; Langendorff preparation; Autonomic innervation; Capsaicin

1. Introduction

Phoneutria nigriventer (armed spider) venom contains polypeptides capable of producing a variety of biological responses, including contraction of vascular smooth muscle (Antunes et al., 1993a), twitching of skeletal muscle (Entwistle et al., 1982), activation of the tissue kallikrein–kinin system (Antunes et al., 1993b; Marangoni et al., 1993; Lopes-Martins et al., 1994) and sensory nerves (Palframan et al., 1996; Costa et al., 1997), blockade of neuromuscular transmission (Souccar et al., 1995), and a delay in the gastric emptying of liquids (Bucaretchi and Collares, 1996). Intravenous administration of dialysed venom evokes a biphasic blood pressure response characterised by a brief hypotension followed by a long-lasting hypertension which are mediated by the activation of adenosine 5′-triphosphate (ATP)-dependent K^+ channels and L-type Ca^{2+} channels, respectively (Costa et al., 1996). To further understand the mechanism by which *P. nigriventer* venom induces systemic hypotension and hypertension, we have investigated the effect of this venom on the isolated rat heart using the Langendorff preparation.

2. Materials and methods

2.1. Langendorff preparation

Male Wistar rats (250–350 g) were anaesthetised with Na^+ pentobarbitone (50 mg/kg, i.p.) and given heparin (500 IU/kg, i.p.) 5 min before thoracotomy. The hearts

* Corresponding author. Fax: +55-19-289-2968; E-mail: iguatu@correionet.com.br

0014-2999/98/$ - see front matter
PII: S0014-2999(98)00767-5

were rapidly excised and mounted on a Langendorff apparatus and perfused at constant pressure (65 mm Hg) with oxygenated (95% 0_2 + 5% $C0_2$) Krebs–Henseleit solution containing (in mM) NaCl 118, KCl 4.7, KH_2P0_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25 and glucose 11, pH 7.4, at 37°C. The left ventricular developed pressure (mm Hg) and heart rate (beats/min) were recorded via a latex balloon inserted into the left ventricle (basal end diastolic pressure ≅ 5 mm Hg) and connected by a polyethylene cannula to a pressure transducer (Ugo Basile, model PRC 21/3) and a two channel recorder (Gemini 7070, Ugo Basile). Coronary flow (ml/min) was measured manually by timed collections of the coronary effluent.

2.2. Reserpine treatment

Adult male Wistar rats were treated with reserpine (5 mg kg^{-1} day^{-1}, i.p., for 2 days) or the corresponding volume (0.5 ml) of vehicle solution (30% (w/v) polyethylene glycol, 1% (v/v) monothioglycerol, 1% (w/v) ascorbic acid, 2% (v/v) benzyl alcohol). The experiments were carried out 7 days after this treatment (Schmidlin et al., 1991).

2.3. Capsaicin desensitisation

Rats were treated subcutaneously (s.c.) with 8-methyl *N*-vanillyl-6-nonenamide (capsaicin; 50 mg/kg) or the corresponding volume (100 μl) of vehicle solution (10% ethanol and 10% Tween 80, in 0.9% (w/v) NaCl solution) on the second day of life, under ether anaesthesia (Jancsó et al., 1977). Sixty to seventy days later, the hearts from these animals were perfused as described above. The efficacy of the capsaicin treatment was assessed in response to capsaicin applied locally to a hind paw rats (Costa et al., 1997). Plasma extravasation was expressed as the plasma volume/100 mg of paw skin by comparison with the level of ^{125}I-albumin present in 100 μl of plasma (Escott and Brain, 1993).

2.4. Experimental protocol

The effects of *P. nigriventer* venom (10, 50 and 100 μg) was investigated in the absence or presence of propranolol (3 μM), CGP-20712A (10 μM), butoxamine (10 μM) or atropine (1 μM). In a separate group of hearts from capsaicin- or reserpine-pretreated rats, the effects of *P. nigriventer* venom were also assessed. The hearts were perfused for 30 min before the administration of venom or test agents such as acetylcholine (20 μg), norepinephrine (0.1 μg) or tyramine (1 mg) used as control, when appropriate.

2.5. Venom and reagents

Since *P. nigriventer* venom (obtained from the Instituto Butantan, São Paulo, Brazil) contains histamine and 5-hydroxytryptamine (5-HT), dialysed venom was used in this study in order to avoid interference by these substances in the bioassay. The venom (10 ml of a 2 mg/ml solution in 0.9% (w/v) NaCl) was dialysed (MW cutoff, 12,000–14,000) for up to 48 h at 4–6°C against 2 l of saline (Antunes et al., 1992).

Acetylcholine, atropine, butoxamine, capsaicin, norepinephrine atropine, propranolol, reserpine, tyramine and dialysis tubing were purchased from Sigma (St. Louis, MO, USA). CGP-20712A was a gift from Dr. Louis A. Barker. ^{125}I-Labelled human serum albumin was purchased from Amersham International (Amersham, UK). Sodium pentobarbitone (Sagatal®) and heparin were obtained from Rhône-Merieux (Dublin, Ireland) and Roche (Rio de Janeiro, Brazil), respectively.

2.6. Statistical analysis

The changes in coronary flow (as %), left ventricular developed pressure and heart rate were evaluated by analysis of variance (ANOVA) followed by Bonferroni's modified *t*-test or Student's paired or unpaired *t*-test as appropriate. The results are shown as the mean ± standard error of the mean (SEM) with $P < 0.05$ considered to indicate significance.

3. Results

3.1. Cardiac changes induced by P. nigriventer venom

Fig. 1 shows that *P. nigriventer* venom (10–100 μg) produced a dose-dependent and reversible rise in left ventricular developed pressure. At a dose of 10 μg ($n = 5$), the venom induced a short-lasting positive inotropic effect (31 ± 9.2% increase in left ventricular developed pressure; $P < 0.05$) and no marked changes in heart rate and coro-

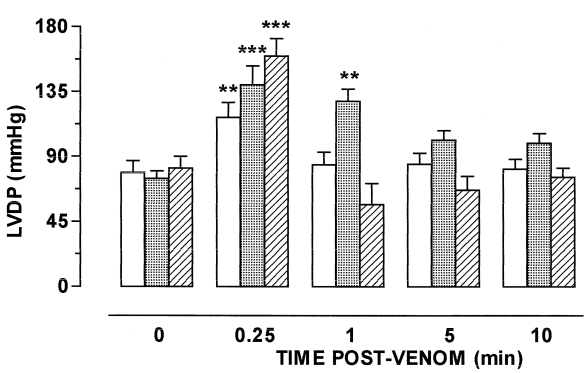

Fig. 1. The increase in left ventricular developed pressure (LVDP) induced by 10 (open bars), 50 (spotted bars) and 100 (stripped bars) μg of *P. nigriventer* venom in isolated rat hearts over 10 min. Each bar represents the mean ± SEM of 5–10 rats. ** $P < 0.01$ and *** $P < 0.001$ compared to their respective basal values (time 0).

nary flow were observed with this dose of venom over a period of 10 min (not shown). At a dose of 50 μg ($n = 10$; Fig. 2A), the venom increased the left ventricular developed pressure by 103 ± 12.5% ($P < 0.001$), and this was followed by occasional ventricular arrhythmia and a modest (but significant) increase in heart rate (Table 1). All of these changes were reversible and the cardiac parameters eventually returned to basal values. Coronary flow was not significantly affected by this dose of venom within 10 min after administration (not shown). At the highest dose (100 μg), the venom produced a progressive and marked decrease in the contractile force of the heart which occurred in two phases. Immediately after venom administration, there was a transitory positive inotropic effect (82 ± 8 mmHg before and 160 ± 12 mm Hg after venom injection; $P < 0.001$, $n = 10$; Fig. 1) associated with prominent bradycardia (Fig. 3). This was then followed by a rapid and transient cardiac arrest, severe rhythm disturbances (1 min post-venom injection) and an increase in the end diastolic pressure at 5 and 10 min post-venom injection (2.2 ± 0.6, 41 ± 6 and 59 ± 12 mmHg before and 5 and 10 min after venom injection, respectively, $n = 10$). There was a simultaneous reduction in coronary flow (Table 2).

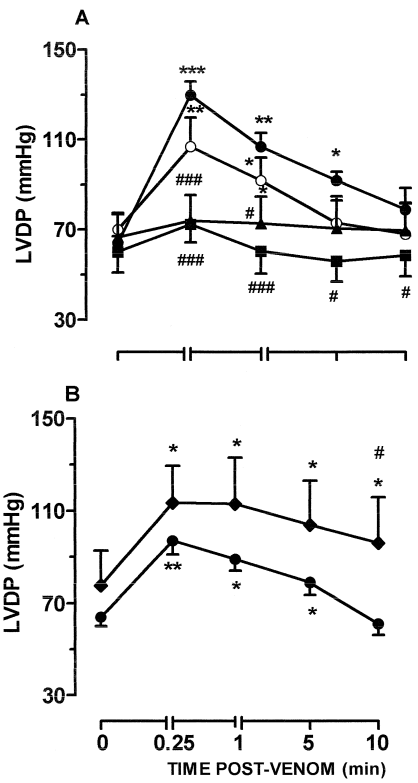

Fig. 2. Changes in the left ventricular developed pressure (LVPD) of isolated rat hearts treated with *P. nigriventer* venom. ●-●, venom alone (50 μg in 100 μl; panels A and B; $n = 10$). The responses to venom in the presence of propranolol (▲-▲, 3 μM; $n = 5$), CGP-20712A (■-■, 10 μM; $n = 7$) or butoxamine (○-○, 10 μM; $n = 6$) are shown in panel A. Panel B shows the response to venom in the presence of atropine (◆-◆, 1 μM; $n = 5$). Each point represents the mean ± SEM of n experiments. $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$ compared to the basal value (time 0). $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ compared to their respective controls.

Table 1
Effect of *P. nigriventer* venom on the heart rate of isolated rat hearts

Treatment	Heart rate (beats/min)		
	Time post-venom (min)		
	Basal	0.25	1
PNV (50 μg)	274 ± 18	305 ± 17[a]	281 ± 15
PNV + propranolol (3 μM)	290 ± 30	300 ± 21	304 ± 40
PNV + CGP-20712A (10 μM)	288 ± 15	276 ± 13	304 ± 10
PNV + butoxamine (10 μM)	296 ± 17	315 ± 19	300 ± 17

The venom (50 μg) was injected in the absence or presence of propranolol (3 μM), CGP-20712A (10 μM) and butoxamine (10 μM). The data are the mean ± SEM of 28 experiments.
[a] $P < 0.05$ compared to the basal value (time 0).

3.2. Effect of the β-adrenoceptor antagonists propranolol, CGP-20712A and butoxamine on the cardiac changes induced by P. nigriventer venom

Continuous infusion of the hearts with propranolol, CGP-20712A or butoxamine had no significant effect on the basal cardiac function. As shown in Fig. 2A and Table 1, the changes in left ventricular developed pressure and heart rate induced by 50 μg of venom were prevented by propranolol (3 μM; $n = 5$) and CGP-20712A (10 μM; $n = 7$). At the same concentrations, propranolol and CGP-20712A also markedly protected against the increase in left ventricular developed pressure, cardiac arrest (Fig. 3C, only propranolol shown) and the reduction in coronary flow (Table 2) induced by 100 μg of venom. The concentrations of propranolol and CGP-20712A employed in this study were effective since they abolished the inotropic and chronotropic responses induced by norepinephrine (0.1 μg, $n = 13$; not shown). In contrast, in butoxamine-treated hearts (10 μM; $n = 6$), the positive inotropic and chronotropic responses to 50 μg of venom were slightly reduced (but not significantly; Fig. 2A, Table 1). Butoxamine treatment failed to prevent the cardiac arrest induced by 100 μg of venom ($n = 6$; not shown), but markedly prevented the reduction in coronary flow induced by 100 μg of venom (Table 2). The increases in left ventricular developed pressure and heart rate induced by norepinephrine (0.1 μg; $n = 6$) were not prevented by butoxamine (not shown).

3.3. Effect of P. nigriventer venom in hearts from reserpine-treated rats

In hearts from vehicle-treated rats, the injection of tyramine (1 mg) caused a significant rise in left ventricular developed pressure (Fig. 4A) which was associated with tachycardia (318 ± 11 before and 402 ± 28 beats/min

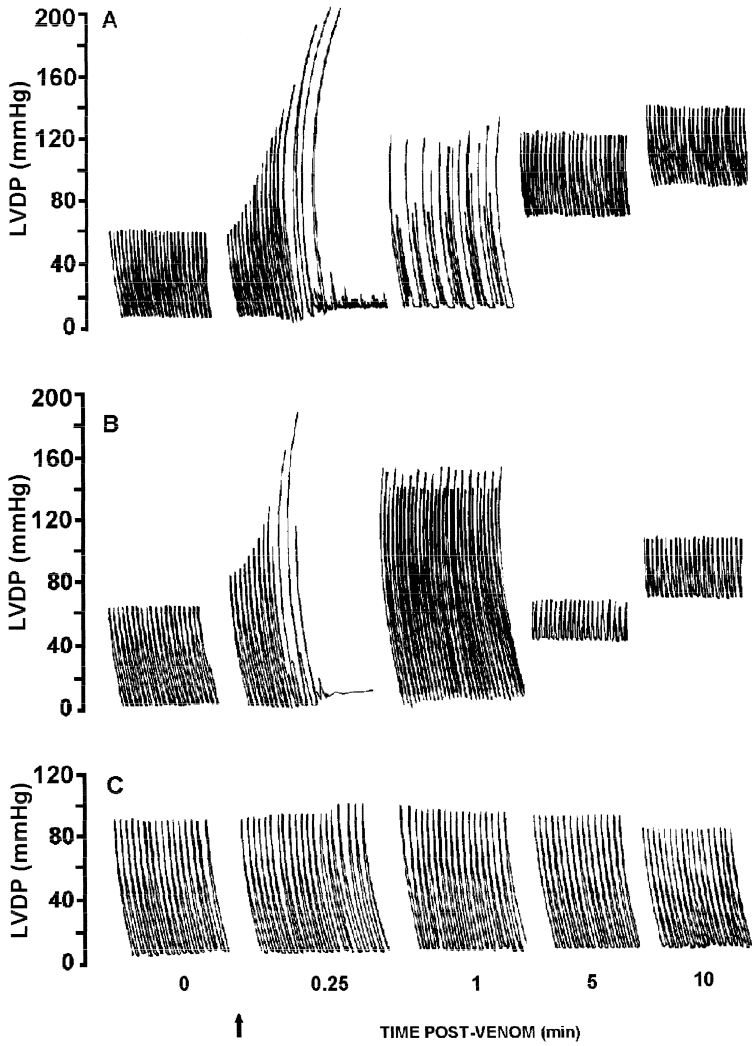

Fig. 3. Changes in the left ventricular developed pressure (LVDP) of isolated rat hearts treated with *P. nigriventer* venom (100 μg). The venom (100 μg; arrow) was injected in the absence (panel A) and presence of atropine (1 μM, panel B) or propranolol (3 μM; panel C). Atropine had no effect on the increase in left ventricular developed pressure, on the cardiac arrest or on the rise in end diastolic pressure, but did prevent the rhythm disturbances observed at 1 min post-venom. Propranolol protected the hearts against all of the venom-induced changes. Each of these traces is representative of 5 to 10 experiments.

1 min post-tyramine injection; $n = 7$, $P < 0.05$). In hearts from reserpine-treated rats ($n = 12$), tyramine (1 mg) failed to significantly affect the left ventricular developed pressure (Fig. 4A) and heart rate (313 ± 24 before and 330 ± 22 beats/min 1 min post-tyramine injection, $n = 12$). The positive inotropic effect caused by norepinephrine in hearts

Table 2
Effect of venom on the coronary flow of isolated rat hearts

Treatment	Changes in coronary flow (%)			
	Time post-venom (min)			
	0.25	1	5	10
PNV (100 μg)	−41 ± 0.8[a]	−36.4 ± 1[a]	−32 ± 0.5[a]	−26.1 ± 0.9[a]
PNV + atropine	−44 ± 0.3[a]	−48 ± 0.6[a]	−24 ± 1[a]	−15 ± 1.1
PNV + propranolol	−6.8 ± 0.6[b]	+7 ± 0.1[b]	+3.4 ± 0.8[b]	−9 ± 1.7[b]
PNV + CGP-20712A	−7.7 ± 1.3[b]	−16 ± 1.7[b]	−17 ± 4.5[b]	−15 ± 8.4
PNV + butoxamine	−14 ± 1.3[b]	−15 ± 2.0[b]	−22 ± 8.0[a]	−12 ± 4.0[b]

The venom (100 μg) was injected in the absence or presence of atropine (1 μM) or the β-adrenoceptor antagonists propranolol (3 μM), CGP-20712A (10 μM) and butoxamine (10 μM). The data represent the mean ± SEM of the % decrease in coronary flow in 28 experiments.
[a] $P < 0.05$ compared to the basal value (100%) of each group.
[b] $P < 0.05$ compared to the venom-alone group in each case.

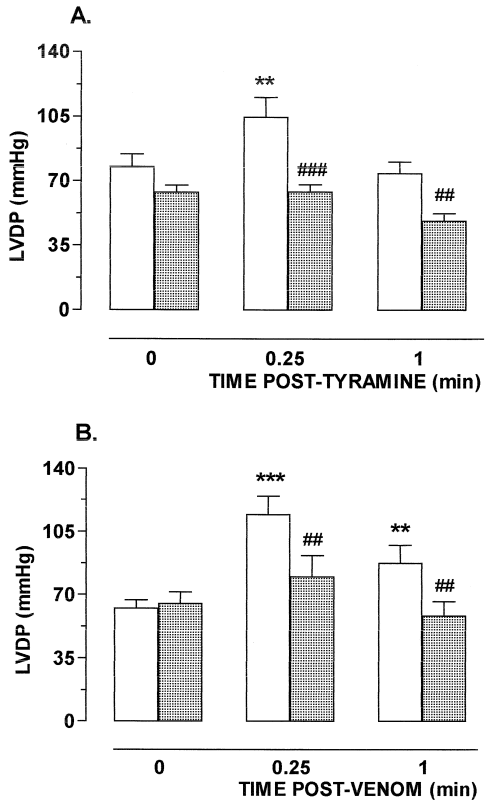

Fig. 4. Inotropic responses to tyramine (1 mg in 100 μl; panel A) and *P. nigriventer* venom (50 μg in 100 μl; panel B) in hearts from vehicle- (open bars; $n = 7$) or reserpine-treated (spotted bars; $n = 12$) rats. Each bar represents the mean ± SEM of n experiments. $* P < 0.05$, $** P < 0.01$ and $*** P < 0.001$ compared to the basal value (time 0). $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ compared to their respective controls.

from reserpine-treated rats (94 ± 4, 108 ± 16 and 142 ± 11 mmHg for 0.01, 0.03 and 0.1 μg, respectively) was significantly higher than in hearts from vehicle-treated rats (84 ± 2, 95 ± 7 and 110 ± 8 mm Hg for 0.01, 0.03 and 0.1 μg, respectively, $n = 7$; $P < 0.05$), thus confirming the expected catecholamine depletion and the supersensitivity of adrenoceptors in those rats treated with reserpine.

The increase in left ventricular developed pressure (see Fig. 4B) and heart rate (273 ± 15 and 315 ± 17 beats/min for basal and 1 min post-venom, respectively, in vehicle-treated hearts; $n = 7$) caused by *P. nigriventer* venom (50 μg) was prevented by treatment with reserpine (280 ± 11 and 255 ± 23 beats/min for basal and 1 min post-venom, respectively, in reserpine-treated hearts; $n = 12$). The incidence of cardiac arrest evoked by 100 μg of venom was greatly diminished (80%) in hearts from reserpine-treated rats ($n = 12$).

3.4. Effect of atropine on the cardiac changes induced by venom

Continuous infusion of the hearts with atropine (1 μM) did not significantly affect the basal cardiac function. At this concentration, atropine significantly potentiated the venom (50 μg)-induced rise in left ventricular developed pressure (Fig. 2B) and the increase in heart rate (8 ± 1.2% for control and 25 ± 3.4% beats/min for atropine-treated hearts 1 min post-venom; $n = 5$). Atropine attenuated the rhythm disturbances observed during 1 min post-venom injection (100 μg) but did not prevent the cardiac arrest (Fig. 3B) or the resulting decrease in coronary flow (Table 2). The dose of atropine used was effective since it abolished the cardiac arrest and the concomitant reduction in coronary flow induced by acetylcholine (20 μg, not shown, $n = 5$).

3.5. Cardiac effects of P. nigriventer venom in hearts from capsaicin-treated rats

As shown in Fig. 5, the venom (50 μg)-induced increase in left ventricular developed pressure in hearts from capsaicin-treated rats ($n = 8$) did not differ significantly from that observed in vehicle-treated rats ($n = 9$). Similarly, the increase in heart rate in the former group was not significantly different from that in the latter (9 ± 0.9% for vehicle and 7.6 ± 1.1% beats/min for capsaicin-treated rat hearts 1 min post-venom). At this dose of venom, no changes in coronary flow were observed (not shown; $n = 9$). All changes in left ventricular developed pressure, heart rate and coronary flow described above for the highest dose of venom (100 μg; $n = 8$) were seen in the capsaicin-treated rats and was not different from that of vehicle-treated group (not shown; $n = 9$). The oedema formation in the hind paw skin in response to capsaicin applied locally to the saphenous nerve was markedly reduced in capsaicin-treated rats (6.2 ± 0.2 μl) compared to the vehicle-treated rats (10.5 ± 0.9 μl; $P < 0.05$), thus confirming the efficacy of the treatment.

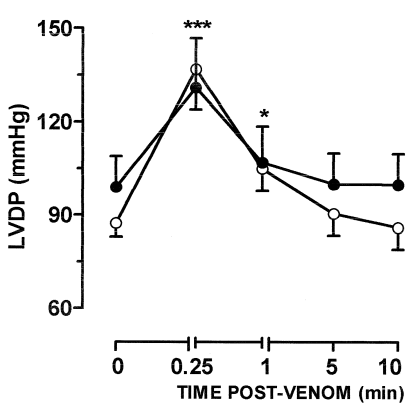

Fig. 5. Changes in the left ventricular developed pressure (LVDP) induced by *Phoneutria nigriventer* venom in isolated hearts from capsaicin-treated rats. The symbols ●-● and ○-○ represent the effects of venom (50 μg in 100 μl) in vehicle- and capsaicin-treated rat hearts, respectively. Each point represents the mean ± SEM of 8 to 9 experiments. $* P < 0.05$ and $*** P < 0.001$ compared to the basal value (time 0).

4. Discussion

Our results clearly demonstrate that *P. nigriventer* venom activates adrenergic autonomic nerve fibers in the isolated rat heart. This activation results in extensive norepinephrine release from sympathetic nerve terminals and, subsequently, in cardiac disturbances characterised mainly by an increase in left ventricular developed pressure and end-diastolic pressure, bradycardia, cardiac arrest and a decrease in coronary flow. This conclusion is based primarily on the observation that propranolol (a non-selective β-adrenoceptor antagonist) and CGP-20712A (a selective β_1-adrenoceptor antagonist) significantly prevented the foregoing cardiac disturbances. In addition, the finding that the cardiac disturbances induced by 50 and 100 μg of venom were substantially attenuated in hearts from reserpine-treated animals further confirms that sympathetic nerve terminals are involved in the cardiac responses. A similar mechanism has been proposed for the delay in gastric emptying following intravenous injection of *P. nigriventer* venom (Bucaretchi and Collares, 1996). Although ventricular muscle contains both functional β_1- and β_2-adrenoceptors (see Brodde, 1991), our results indicate a major role for the former on the cardiac disturbances induced by this venom. Interestingly, the muscarinic receptor antagonist atropine slightly potentiated the venom-induced increase in left ventricular developed pressure and attenuated the rhythm disturbances, suggesting that the venom also releases small amounts of acetylcholine from parasympathetic nerve terminals. Although these results are unclear, they may reflect the presence of muscarinic receptors in sympathetic nerves at presynaptic sites which once activated lead to inhibition of norepinephrine release (Muscholl, 1980; Löffelholz and Pappano, 1985). Our data are therefore consistent with the suggestion that atropine acts indirectly by antagonizing muscarinic receptors in sympathetic nerves at the presynaptic level, thereby further enhancing norepinephrine release.

The mammalian heart is innervated by both inhibitory cholinergic and excitatory adrenergic autonomic nerve fibers. Cholinergic innervation occurs mainly in the sinoatrial and atrioventricular nodes and is less abundant in the bundle of His and Purkinje fibers. Adrenergic innervation is widely distributed throughout the myocardium (Shavit et al., 1986; Hoover and Hancock, 1988). In guinea-pig isolated auricles, undialysed *P. nigriventer* venom activates voltage-sensitive Na^+ channels resulting in the release of both acetylcholine and norepinephrine from the autonomic terminals of this preparation (Vital Brazil et al., 1988). However, in the Langendorff preparation used in our experiments, the venom appears to have a preferential action on adrenergic fibers. It is possible that the large amounts of acetylcholine released from guinea-pig auricles by the venom (Vital Brazil et al., 1988) reflects the predominantly parasympathetic innervation of this preparation. Alternatively, the difference between these two animal species may be due to the presence of histamine and serotonin in undialysed venom, both of which are known to affect the cardiac inotropic and chronotropic responses (Endou et al., 1994; Molderings et al., 1996). The activation of both H_3 histaminergic and serotoninergic receptors located in sympathetic fibers at presynaptic sites also inhibits norepinephrine release (Muscholl, 1980; Löffelholz and Pappano, 1985; Endou et al., 1994; Molderings et al., 1996) and this could contribute to the differences observed between undialysed and dialysed venom in rats and guinea-pigs. Undialysed venom also contains a number of low molecular weight peptides (< 10 kDa; Rezende et al., 1991), some of which are able to activate or inhibit different types of ionic channels (Romano-Silva et al., 1993, 1996; Troncone et al., 1995) and could possibly affect cardiac function.

Oedema formation induced by the intradermal injection of *P. nigriventer* venom in rat skin is partially inhibited by the NK_1 tachykinin receptor antagonist, SR140333 (Palframan et al., 1996) and substantially attenuated in capsaicin-pretreated rats (Costa et al., 1997), indicating that the venom contains components which directly stimulate sensory nerve terminals to release vasoactive mediators including calcitonin gene-related peptide (CGRP) and substance P. CGRP exerts potent cardiostimulatory effects in heart preparations in vitro (Tippins et al., 1984; Franco-Cereceda et al., 1988; Gepetti et al., 1988), whereas substance P causes bradycardia in isolated guinea-pig hearts by a mechanism involving the stimulation of intrinsic cholinergic neurones localised in epicardial ganglia near the origin of the ascending aorta and pulmonary trunk, as well as at other sites (Hoover and Hancock, 1988; Hoover, 1989). The failure of atropine to affect the venom-induced bradycardia excludes the above mechanism. Furthermore, the finding that the venom-induced cardiac disturbances were unchanged in hearts from capsaicin-treated rats provides further evidence that neither the positive inotropic and chronotropic effects nor the bradycardia induced by this venom are due to the local release of CGRP or substance P, respectively. The capsaicin treatment used in this study was effective since it reduced the capsaicin-induced oedema in the rat hind paw, as previously described (Costa et al., 1997). In addition, similar treatment with capsaicin leads to a degeneration of sensory nerves within the heart and the subsequent depletion of neuropeptides such as CGRP and substance P (Papka et al., 1984; Wharton et al., 1986; Franco-Cereceda and Lundberg, 1988).

Cardiac disturbances such as tachycardia and arrhythmia are frequently observed in envenomation induced by *P. nigriventer* (Lucas, 1988). In contrast to our observations in the isolated hearts, the venom-induced hypertension in vivo is not prevented by either propranolol or the α adrenoceptor antagonist phenoxybenzamine (Costa et al., 1996). This discrepancy probably reflects the fact that venom-induced haemodynamic changes are of a cen-

tral origin and unrelated to sympathetic system activation (unpublished observations). The venom-induced hypertension is preceded by a brief hypotension which is partially mediated by the activation of ATP-dependent K^+ channels (Costa et al., 1996). Our results in vitro showed that bradycardia and a reduction in coronary flow were observed only at a high dose of venom (100 μg) which severely altered cardiac function. These findings suggest that multiple mechanisms contribute to venom-induced systemic hypotension and may not necessarily involve alterations in cardiac function. In conclusion, our data show that *P. nigriventer* venom predominantly activates cardiac sympathetic nerve endings, leading to norepinephrine release.

Acknowledgements

S.K.P. Costa is the recipient of a studentship from FAPESP (Brazil). S.D. Brain and G. de Nucci thank the Wellcome Trust for financial support.

References

Antunes, E., Marangoni, R.A., Brain, S.D., de Nucci, G., 1992. *Phoneutria nigriventer* (armed spider) venom induces increased vascular permeability in rat and rabbit skin in vivo. Toxicon 30, 1011–1016.

Antunes, E., Marangoni, R.A., Borges, N.C.C., Hyslop, S., Fontana, M.D., de Nucci, G., 1993a. Effects of *Phoneutria nigriventer* venom on rabbit vascular smooth muscle. Braz. J. Med. Biol. Res. 26, 81–91.

Antunes, E., Marangoni, R.A., Giglio, J.R., Brain, S.D., de Nucci, G., 1993b. Activation of tissue kallikrein–kininogen–kinin system in rabbit skin by a fraction isolated from *Phoneutria nigriventer* (armed spider) venom. Toxicon 31, 1385–1391.

Brodde, O.E., 1991. β_1 and β_2 adrenoceptors in the human heart: properties, function and alterations in chronic heart failure. Pharmacol. Rev. 43, 203–242.

Bucaretchi, F., Collares, E.F., 1996. Effect of *Phoneutria nigriventer* spider venom on gastric emptying in rats. Braz. J. Med. Biol. Res. 29, 205–211.

Costa, S.K.P., Moreno, H. Jr., Brain, S.D., de Nucci, G., Antunes, E., 1996. The effect of *Phoneutria nigriventer* (armed spider) venom on arterial blood pressure of anaesthetised rats. Eur. J. Pharmacol. 298, 113–120.

Costa, S.K.P., Antunes, E., de Nucci, G., Brain, S.D., 1997. *Phoneutria nigriventer* spider venom induces oedema in rat skin by activation of capsaicin sensitive sensory nerves. Eur. J. Pharmacol. 339, 223–226.

Endou, M., Poli, E., Levi, R., 1994. Histamine H_3-receptor signaling in the heart: possible involvement of Gi/Go proteins and N-type Ca^{2+} channels. J. Pharmacol. Exp. Ther. 269, 221–229.

Entwistle, I.D., Johnstone, R.A.W., Medzihradszky, D., May, T.E., 1982. Isolation of a pure toxic polypeptide from the venom of the spider *Phoneutria nigriventer* and its neurophysiological activity on an insect femur preparation. Toxicon 20, 1059–1067.

Escott, K.J., Brain, S.D., 1993. Effect of a calcitonin gene-related peptide antagonist ($CGRP_{8-37}$) on skin vasodilatation and oedema induced by stimulation of the rat saphenous nerve. Br. J. Pharmacol. 110, 772–776.

Franco-Cereceda, A., Lundberg, J.M., 1988. Actions of calcitonin gene-related peptide and tachykinins in relation to the contractile effects of capsaicin in the guinea-pig and rat heart in vitro. Naunyn-Schmiedeberg's Arch. Pharmacol. 337, 649–655.

Franco-Cereceda, A., Lundberg, J.M., Saria, A., Schreibmayer, W., Tritthart, H.A., 1988. Calcitonin gene-related peptide: release by capsaicin and prolongation of the action potential in the guinea-pig heart. Acta Physiol. Scand. 132, 181–190.

Gepetti, P., Maggi, C.A., Perreti, F., Frilli, S., Manzini, S., 1988. Simultaneous release of bradykinin, substance P, and calcitonin gene-related peptide immunoreactivities from capsaicin sensitive structures in guinea-pig heart. Br. J. Pharmacol. 94, 288–290.

Hoover, D.B., 1989. Effects of substance P on rate and perfusion pressure in the isolated guinea-pig heart. J. Pharmacol. Exp. Ther. 252, 179–184.

Hoover, D.B., Hancock, J.C., 1988. Distribution of substance P binding sites in guinea-pig heart and pharmacological effects of substance P. J. Auton. Nerv. Syst. 23, 189–197.

Jancsó, G., Kiraly, E., Jansco-Gabor, A., 1977. Pharmacologically-induced selective degeneration of chemosensitive primary sensory neurones. Nature 270, 741–742.

Lopes-Martins, R.A.B., Antunes, E., Oliva, M.L.V., Sampaio, C.A.M., Burton, J., de Nucci, G., 1994. Pharmacological characterisation of rabbit corpus cavernosum relaxation mediated by the tissue kallikrein–kinin system. Br. J. Pharmacol. 113, 81–86.

Löffelholz, K., Pappano, A.J., 1985. The parasympathetic neuroeffector junction of the heart. Pharmacol. Rev. 37, 1–24.

Lucas, S., 1988. Spiders in Brazil. Toxicon 26, 759–772.

Marangoni, R.A., Antunes, E., Brain, S.D., de Nucci, G., 1993. Activation by *Phoneutria nigriventer* (armed spider) venom of tissue kallikrein–kininogen–kinin system in rabbit skin in vivo. Br. J. Pharmacol. 109, 539–543.

Molderings, G.J., Frölich, D., Likungu, J., Göthert, M., 1996. Inhibition of noradrenaline release via presynaptic $5\text{-}HT_{1D}\alpha$ receptors in human atrium. Naunyn-Schmiedeberg's Arch. Pharmacol. 353, 272–280.

Muscholl, E., 1980. Peripheral muscarinic control of norepinephrine release in the cardiovascular system. Am. J. Physiol. 239, H713–H720.

Palframan, R., Wilsoncroft, P., Costa, S.K.P., Antunes, E., de Nucci, G., Brain, S.D., 1996. The effect of a tachykinin NK_1 receptor antagonist SR140333 on oedema formation induced in rat skin by venom from the spider *Phoneutria nigriventer*. Br. J. Pharmacol. 118, 295–298.

Papka, R.E., Furness, J.B., Della, N.G., Murphy, R., Costa, R., 1984. Time course of effect of capsaicin on ultrastructural and histochemistry of substance P-immunoreactive nerves associated with the cardiovascular tissue of the rat. Neuroscience 12, 1277–1292.

Rezende, L. Jr., Cordeiro, M.N., Oliveira, E.B., Diniz, C.R., 1991. Isolation of neurotoxic peptides from the venom of the armed spider *Phoneutria nigriventer*. Toxicon 29, 1225–1233.

Romano-Silva, M.A., Ribeiro-Santos, R., Ribeiro, A.M., Gomez, M.V., Diniz, C.R., Cordeiro, M.N., Brammer, M.J., 1993. Rat cortical synaptosomes have more than one mechanism for Ca^{2+} entry linked to rapid glutamate release: studies using the *Phoneutria nigriventer* toxin PhTX2 and potassium depolarization. Biochem. J. 296, 313–319.

Romano-Silva, M.A., Gomez, M.V., Diniz, C.R., Cordeiro, M.N., Ribeiro, A.M., 1996. Acetylcholine release from rat brain cortical slices evoked by the fraction P4 of the venom of the spider *Phoneutria nigriventer* has Ca^{2+} and temperature independent components. Neurosci. Lett. 219, 159–162.

Schmidlin, O., Garcia, J., Schwartz, J.B., 1991. The effects of aging on the eletrophysiologic responses to verapamil in isolated perfused rat hearts. J. Pharmacol. Exp. Ther. 258, 130–134.

Shavit, G., Gitter, S., Barak, Y., Vidne, B.A., Oron, Y., 1986. Positive inotropic response to alpha-adrenergic stimulation in electrically-driven rat left atrium: the role of external Ca^{2+}. J. Cardiovasc. Pharmacol. 8, 324–335.

Souccar, C., Gonçalo, M.C., Lapa, A.J., Troncone, L.R.P., Lebrun, I., Magnoli, F., 1995. Blockade of acetylcholine release at the motor endplate by a polypeptide from the venom of *Phoneutria nigriventer*. Br. J. Pharmacol. 116, 2817–2823.

Tippins, J.R., Morris, H.R., Pamco, M., Etienne, T., Bevis, P., Girgis, S., MacIntyre, I., Azria, M., Attinger, M., 1984. The myotropic and

plasma-Ca^{2+} modulating effects of calcitonin gene-related peptide (CGRP). Neuropeptides 4, 425–434.

Troncone, L.R., Lebrun, I., Magnoli, F., Yamane, T., 1995. Biochemical and pharmacological studies on a lethal neurotoxic polypeptide from *Phoneutria nigriventer* spider venom. Neurochem. Res. 20, 879–883.

Vital Brazil, O., Leite, G.B., Fontana, M.D., 1988. Modo de ação da peçonha da aranha armadeira, *Phoneutria nigriventer* (Keyserling, 1891), nas aurículas isoladas de cobaia. Ciênc. Cult. S Paulo 40, 181–185.

Wharton, J., Gulbienkian, S., Mulderry, P.K., Ghatei, M.A., McGregor, G.P., Bloom, S.R., Plak, J., 1986. Capsaicin induces a depletion of calcitonin gene-related peptide (CGRP)-immunoreactivity in the cardiovascular system of the guinea-pig and rat. J. Auton. Nerv. Syst. 16, 289–309.