ELSEVIER

European Journal of Pharmacology 298 (1996) 113–120

ejp

The effect of *Phoneutria nigriventer* (armed spider) venom on arterial blood pressure of anaesthetised rats

Soraia K.P. Costa [a], Heitor Moreno Jr. [a], Susan D. Brain [b], Gilberto De Nucci [a], Edson Antunes [a,*]

[a] *Department of Pharmacology, Faculty of Medical Sciences, UNICAMP, P.O. Box 6111, 13081-970 Campinas (SP), Brazil*
[b] *Pharmacology Group, Biomedical Sciences Division, King's College, Manresa Rd, London, SW3 6LX, UK*

Received 16 October 1995; accepted 31 October 1995

Abstract

The changes induced in the mean arterial blood pressure of anaesthetised rats following the administration of armed spider (*Phoneutria nigriventer*) venom have been investigated. The intravenous injection of *Phoneutria nigriventer* venom (0.1 mg/kg) evoked a brief and reversible decrease in the mean arterial blood pressure whereas a higher dose of venom (0.3 mg/kg) caused a biphasic response characterized by a short-lasting hypotension followed by a sustained and prolonged hypertension (40–50 min). These changes were accompanied by tachycardia, salivation, fasciculations, defecation and respiratory disturbances. Pretreatment of the animals with atropine (10 mg/kg), propranolol (100 mg/kg), phenoxybenzamine (100 mg/kg) and indomethacin (4 mg/kg) did not significantly affect the mean arterial blood pressure changes induced by *Phoneutria nigriventer* venom. Similarly, the bradykinin B_2 receptor antagonist Hoe 140 (D-Arg-[Hyp^3,Thi^5,dTic^7,Oic^8]-bradykinin) (0.6 mg/kg), the PAF receptor antagonist WEB 2086 (3-(4-(2-chlorophenyl)-9-methyl-6*H*-thieno-(3,2*f*) (1,2,4)-triazolo-(4,3-*a*) (1,4)-diazepine-2-yl)-(4-morpholinyl)-1-propanone) (20 mg/kg), the tachykinin NK_1 receptor antagonist SR 140333 ((*S*)1-{2-[3-(3,4-dichlorophenyl)-1-(3-iso-propoxyphenyl acetyl) piperidin-3-yl] ethyl}-4-phenyl-1-azoniabicyclo[2.2.2] octane, chloride) (0.5 mg/kg), the tachykinin NK_2 receptor antagonist SR 48968 ((*S*)-*N*-methyl-*N*[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butyl]benzamide) (0.5 mg/kg) and the nitric oxide synthase inhibitor N^{ω}-nitro-L-arginine methyl ester (10 mg/kg) had no significant effect on the mean arterial blood pressure changes induced by *Phoneutria nigriventer* venom. The increase in the blood pressure induced by *Phoneutria nigriventer* venom was also not significantly affected by either the angiotensin II receptor antagonist losartan (10 mg/kg) or the endothelin ET_A receptor antagonist FR 139317 ((*R*)2-[(*R*)-2-[[1-(hexahydro-1*H*-azepinyl]carbonyl]amino-4-methyl-pentanoyl]amino-3-[3-(1-methyl-1*H*-indoyl)]propionyl] amino-3-(2-pyridyl) propionic acid) (30 mg/kg). The ATP-dependent K^+ channel antagonist glibenclamide (50 mg/kg) reduced by 40% the hypotension induced by *Phoneutria nigriventer* venom without affecting the hypertensive response. Pretreatment of the animals with L-type Ca^{2+} channel antagonists such as verapamil (10–100 μg/kg/min), diltiazem (40–120 μg/kg/min) and nifedipine (0.3–10 mg/kg) markedly attenuated the hypertension induced by *Phoneutria nigriventer* venom. Verapamil (30 μg/kg/min) and diltiazem (120 μg/kg/min) also promptly reversed the established hypertension induced by *Phoneutria nigriventer* venom when infused 8 min after venom injection. Our results indicate that the brief decrease of blood pressure induced by *Phoneutria nigriventer* venom is partially due to ATP-dependent K^+ channel activation. The prolonged hypertension seems to result from direct Ca^{2+} entry into vascular and/or cardiac muscles.

Keywords: Spider venom; Ca^{2+} channel blocker; K^+ channel blocker; Endothelin-1; Nitric oxide (NO); Hoe 140

1. Introduction

Human envenomation by the spider *Phoneutria nigriventer* is common in South America, particularly in Brazil (Lucas, 1988). The predominant clinical manifestations of such envenomation include intense local pain, visual disturbances, priapism, neurogenic shock, tachycardia and arrhythmias (Brazil and Vellard, 1925).

Phoneutria nigriventer venom activates voltage-dependent sodium channels in both the phrenic nerve-diaphragm muscle preparation (Fontana and Vital-Brazil, 1985) and in guinea pig auricles (Vital-Brazil et al., 1988), but not in rabbit isolated vascular smooth muscle (Antunes et al., 1993a). In the auricles, *Phoneutria nigriventer* venom releases both acethylcholine and norepinephrine (Vital-

* Corresponding author. Fax: 55 192 52 1516.

Elsevier Science B.V.
SSDI 0014-2999(95)00739-3

Brazil et al., 1988) which are believed to play a role in the cardiac disturbances observed following envenomation by this species (Schenberg and Pereira-Lima, 1971). Since the in vivo cardiovascular effects of *Phoneutria nigriventer* venom have not been systematically studied, the aim of the present work was to investigate the effects of *Phoneutria nigriventer* venom on the arterial blood pressure of anaesthetised rats.

2. Materials and methods

2.1. Arterial blood pressure measurement

Male Wistar rats (280–350 g) provided by CEMIB-UNICAMP were anaesthetised with sodium pentobarbital (Sagatal, 40 mg/kg i.p.) and the trachea was cannulated. The right carotid artery and left femoral vein were cannulated for the measurement of arterial bood pressure and drug administration, respectively. The arterial pressure was measured via a pressure transducer (Ugo Basile, model PRC 21/3) connected to a two-channel polygraph (Ugo Basile). The experiments were initiated after at least 15 min of stabilization at which point the arterial blood pressure was generally 80–100 mm Hg. *Phoneutria nigriventer* venom and the other agonists (noradrenaline, isoproterenol, acetylcholine, bradykinin, platelet-activating factor, angiotensin II, endothelin-1, substance P, cromakalim, sodium nitroprusside and Bay K8644 (methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate) employed in this study were injected intravenously as a single bolus (100 μl) and were washed in with a further 100 μl of saline.

2.2. Dialysis of Phoneutria nigriventer venom

Phoneutria nigriventer venom (10 ml of a 2 mg/ml of 0.9% w/v saline solution) was dialysed (dialysis membrane molecular weight cutoff, 12000–14000) for up to 48 h at 4–6°C against 2 l of saline. The dialysing solution was changed 4 times during this period (Antunes et al., 1992).

2.3. Venom and reagents

Lyophilised *Phoneutria nigriventer* venom was supplied by the Butantan Institute (São Paulo, Brazil). The venom was collected from the spiders by means of electrical stimulation and desiccated in vacuo over NaOH tablets at room temperature.

Acetylcholine, angiotensin II, atropine, bradykinin, diltiazem, indomethacin, isoproterenol, N^{ω}-nitro-L-arginine methyl ester, noradrenaline, propranolol, verapamil, glibenclamide, cromakalim, substance P, sodium nitroprusside and dialysis tubing (molecular weight cutoff 12000–14000) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Platelet-activating factor (PAF) and sodium pentobarbital (Sagatal) were obtained from Beecham (Bubendorf, Switzerland) and May & Baker (UK), respectively. WEB 2086 and phenoxybenzamine were obtained from SmithKline Beecham (UK). Losartan was supplied by Merck (USA). Endothelin-1 was supplied by the Peptide Institute (Osaka, Japan). The endothelin (ET_A) receptor antagonist FR 139317 ((*R*)2-[(*R*)-2-[[1-(hexahydro-1*H*-azepinyl]carbonyl]amino-4-methyl-pentanoyl]amino-3-[3-(1-methyl-1*H*-indoyl)]propionyl] amino-3-(2-pyridyl) propionic acid) was supplied by Dr. T.J. Opgenorth (Abbott Laboratories, IL, USA). Hoe 140 (D-

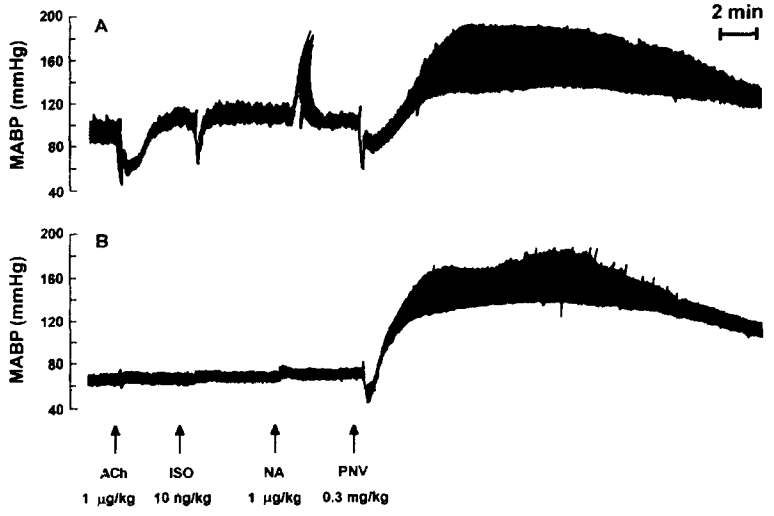

Fig. 1. A typical trace of the mean arterial blood pressure (MABP) induced by acetylcholine (ACh, 1 μg/kg i.v.), isoproterenol (ISO, 10 ng/kg i.v.), noradrenaline (NA, 1 μg/kg i.v.) and *Phoneutria nigriventer* venom (PNV, 0.3 mg/kg i.v.) in an animal receiving saline (panel A) and in an animal pre-treated 0.5 h before with a mixture of atropine (10 mg/kg i.p.), propranolol (100 mg/kg i.p.) and phenoxybenzamine (100 mg/kg i.p.; panel B).

Arg-[Hyp^3,Thi^5,DTic^7,Oic^8]-bradykinin), BAY K8644 and nifedipine were obtained from Pharma Synthesis (Hoechst), Bayer (Germany) and Bayer (Brazil), respectively. SR 140333 ((*S*)1-{2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxyphenyl acetyl) piperidin-3-yl] ethyl}-4-phenyl-1-azoniabicyclo[2.2.2] octane, chloride) and SR 48968 ((*S*)-*N*-methyl-*N*[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butyl]benzamide) were supplied by Sanofi Recherche (Montpellier, France). FR 139317 was dissolved in 1 N NaOH and the pH corrected to 7.4. BAY K 8644 was dissolved in 40% ethanol. *Phoneutria nigriventer* venom and test agents were stored in stock solutions at −20°C and then diluted with isotonic saline (0.9% w/v) prior to administration to the animals.

2.4. Statistical analysis

The results are presented as the mean ± S.E.M. where appropriate. Statistical comparison was undertaken by means of analysis of variance (ANOVA) followed by either Student's unpaired two-tailed *t*-test or Duncan's multiple range test. Values of $P < 0.05$ were considered as significant.

3. Results

3.1. The effects of Phoneutria nigriventer venom on mean arterial blood pressure

The intravenous administration of *Phoneutria nigriventer* venom (0.1 mg/kg) caused a brief (2–4 min) and reversible hypotension (43 ± 7 mm Hg, $n = 7$) whereas a higher venom dose (0.3 mg/kg) induced a biphasic mean arterial blood pressure response consisting of a brief (1–3 min) hypotension (33 ± 2.2 mm Hg) followed by a prolonged hypertension (42.5 ± 4.8 mm Hg increase; Fig. 1, upper panel). The latter phase was characterised by a slow onset (10 min to reach the maximal response), long duration (approximately 40–50 min) and associated with a discrete increase in the heart rate (496 ± 12 beats/min before and 544 ± 8.5 beats/min 10 min after *Phoneutria nigriventer* venom injection, $n = 10$, $P < 0.05$). A second administration of *Phoneutria nigriventer* venom (0.3 mg/kg) always evoked a tachyphylactic response followed by death in 75% of the animals 10 min after injection ($n = 20$). The changes in blood pressure induced by this dose of venom were accompanied by intense salivation, fasciculations and defecation.

3.2. Effect of atropine, phenoxybenzamine and propranolol

The intravenous injection of both acetylcholine (1 μg/kg) and the β-adrenoceptor agonist isoproterenol (10 ng/kg) induced a short-lasting hypotension (61 ± 6.2 and 39.6 ± 2.9 mm Hg, respectively, $n = 5$) whereas noradrenaline (1 μg/kg) induced a brief hypertension (53.4 ± 5.3 mm Hg). Pretreatment of one group of rats with the muscarinic receptor antagonist atropine (10 mg/kg i.p., 0.5 h before) significantly reduced the acetylcholine-induced hypotension without affecting the biphasic blood pressure response induced by *Phoneutria nigriventer* venom (0.3 mg/kg; Fig. 2A). At this dose, atropine did not significantly change basal blood pressure (103 ± 7.5 and 83.5 ± 4.7 mm Hg for control and atropine-treated animals, respectively, $n = 5$). In a second group of rats, pretreatment with the β-adrenoceptor antagonist propranolol (100 mg/kg i.p., 0.5 h before) did not change the basal mean arterial blood pressure (101.2 ± 5.6 and 95.6 ± 7.3 mm Hg for control and propranolol-treated animals, respectively, $n = 5$) but markedly reduced the isoproterenol-induced hypotension (Fig. 2B). Propranolol treatment did not significantly affect the blood pressure changes induced by *Phoneutria nigriventer* venom. Finally, in a third group of rats, pretreatment with the α-adrenoceptor antagonist phenoxybenzamine (100 mg/kg i.p., 0.5 h before) lowered the basal blood pressure (114 ± 2.3 and 78 ± 2.2 mm Hg for control and phenoxybenzamine-treated animals, respectively, $n = 5$, $P < 0.05$) and significantly reduced the noradrenaline-induced hypertension (Fig. 2C).

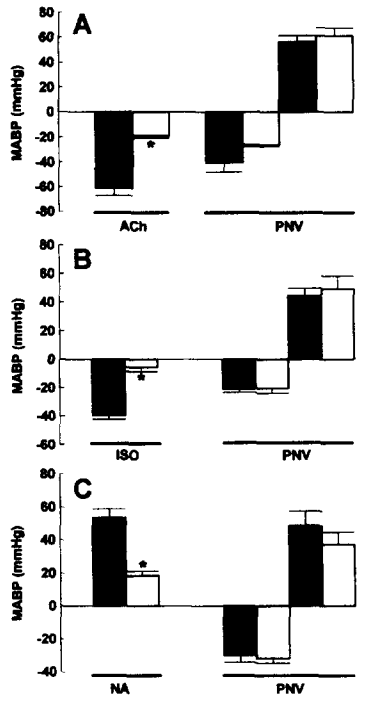

Fig. 2. The effect of atropine (panel A; 10 mg/kg i.p., 0.5 h before), propranolol (panel B; 100 mg/kg i.p., 0.5 h before) and phenoxybenzamine (panel C; 100 mg/kg i.p., 0.5 h before) on the arterial blood pressure changes induced by *Phoneutria nigriventer* venom (PNV; 0.3 mg/kg i.v.) in anaesthetised rats. Acetylcholine (ACh, 1 μg/kg i.v.), isoproterenol (ISO, 10 ng/kg i.v.) and noradrenaline (NA, 1 μg/kg i.v.) were injected as controls for the antagonists described above. Closed bars represent the control (saline-treated) animals and the open bars represent the drug-treated animals. Bars represent the means ± S.E.M. * $P < 0.05$ compared to the control values.

Table 1
Effect of Hoe 140 (0.6 mg/kg i.p.), WEB 2086 (20 mg/kg i.v.), N^{ω}-nitro-L-arginine methyl ester (L-NAME; 10 mg/kg i.v.), losartan (10 mg/kg i.v), FR 139317 (30 mg/kg i.v.) and SR 140333 (0.5 mg/kg i.v.) on the mean arterial blood pressure (MABP) changes induced by *Phoneutria nigriventer* venom (PNV; 0.3 mg/kg)

Treatment	MABP decrease (mm Hg)	MABP increase (mm Hg)	n
PNV	43.6 ± 5.7	48.0 ± 5.7	5
PNV + Hoe 140	45.2 ± 9.6	55.0 ± 7.1	5
PNV	36.0 ± 9.0	42.4 ± 9.9	5
PNV + WEB 2086	49.6 ± 7.1	29.0 ± 8.2	5
PNV	36.0 ± 8.5	42.4 ± 10.0	5
PNV + L-NAME	43.8 ± 7.3	34.6 ± 7.3	5
PNV	31.2 ± 4.6	51.4 ± 8.5	5
PNV + losartan	15.6 ± 2.2 [a]	57.6 ± 5.4	5
PNV	33.0 ± 2.2	42.5 ± 4.8	7
PNV + FR 139317	36.8 ± 4.7	43.4 ± 3.9	7
PNV	25.2 ± 2.6	46.2 ± 11.6	6
PNV + SR 140333	22.0 ± 2.0	40.5 ± 5.2	6

The values are the mean ± S.E.M. of n rats. [a] $P < 0.05$ as compared to the control (PNV alone) group.

At this dose, phenoxybenzamine had no effect on the blood pressure changes induced by *Phoneutria nigriventer* venom.

When the animals were previously treated with a mixture of atropine, propranolol and phenoxybenzamine (same doses as described above), the mean arterial blood pressure decreased from 100.3 ± 4.0 to 69.0 ± 3.7 mm Hg ($n = 5$, $P < 0.05$). This treatment virtually abolished the responses induced by acetylcholine, isoproterenol and noradrenaline without significantly affecting the blood pressure changes induced by *Phoneutria nigriventer* venom (Fig. 1, lower panel).

3.3. Effect of the cyclooxygenase inhibitor indomethacin

Pretreatment of the animals with indomethacin (4 mg/kg i.p., 0.5 h before) affected neither the hypotension (33.0 ± 2.2 and 36.0 ± 6.0 mm Hg for control and treated animals, respectively) nor the hypertension (42.5 ± 4.8 and 39.8 ± 3.7 mm Hg for control and treated animals, respectively, $n = 5$) induced by *Phoneutria nigriventer* venom. Indomethacin did not affect the basal blood pressure (112.4 ± 7.4 and 102.3 ± 5.9 mm Hg for control and indomethacin-treated animals, respectively, $n = 5$).

3.4. Effect of the bradykinin B_2 receptor antagonist Hoe 140 and the PAF receptor antagonist WEB 2086

The intravenous bolus administration of bradykinin (1 μg/kg) caused a short-lived hypotension (22.5 ± 4.3 mm Hg) which was markedly reduced (1.6 ± 1.8, $n = 5$, $P < 0.01$) when the animals were pretreated with Hoe 140 (0.6 mg/kg i.p.). However, Hoe 140 failed to significantly affect mean arterial blood pressure changes induced by *Phoneutria nigriventer* venom (Table 1). Similarly, WEB 2086 (20 mg/kg i.v., $n = 5$) abolished PAF (0.25 μg/kg i.v.)-induced hypotension (37.8 ± 7.4 mm Hg, $n = 5$; $P < 0.01$) without significantly affecting blood pressure changes induced by *Phoneutria nigriventer* venom (Table 1). At the doses used, Hoe 140 and WEB 2086 did not affect the basal blood pressure (not shown).

3.5. Effect of tachykinin NK_1 (SR 140333) and NK_2 (SR 48968) receptor antagonists

Intravenous bolus injection of subtance P (1 μg/kg) caused a brief hypotension (29.3 ± 2.0 mm Hg, $n = 6$) which was abolished when the animals were previously treated with SR 140333 (0.5 mg/kg i.v, 10 min before). However, this antagonist (same dose) had no effect on the blood pressure changes induced by *Phoneutria nigriventer* venom ($n = 6$; Table 1). Pretreatment of the animals with SR 48968 (0.5 mg/kg i.v., 10 min before) affected neither the hypotension induced by substance P nor the the blood pressure changes induced by *Phoneutria nigriventer* venom ($n = 6$; not shown). SR 140333 and SR 48968 did not affect the basal blood pressure (not shown).

3.6. Effect of the nitric oxide synthesis inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME)

The administration of L-NAME (10 mg/kg i.v.) caused a sustained elevation of mean arterial blood pressure (25.0 ± 4.5% increase, $n = 5$, $P < 0.01$). In the N^{ω}-nitro-L-arginine methyl ester-treated animals, the blood pressure changes induced by *Phoneutria nigriventer* venom were not significantly different from that observed in animals receiving saline (Table 1).

Table 2
Reversal by verapamil and diltiazem of the established hypertension induced by *Phoneutria nigriventer* venom (PNV; 0.3 mg/kg i.v.)

Treatment	MABP (mm Hg)		
	Basal	Maximal hypertension by PNV	8 min after Ca^{2+} antagonists
Saline (25 μl/min)	98.0 ± 6.0	145.0 ± 5.0 [a]	139.0 ± 3.0
Verapamil (30 μg/kg/min)	106.0 ± 3.7	138.0 ± 6.6 [a]	88.8 ± 4.6 [b]
Diltiazem (120 μg/kg/min)	88.5 ± 5.9	131.5 ± 10.0 [a]	89.5 ± 6.7 [b]

Verapamil and diltiazem were intravenously infused 5 min after PNV administration. MABP, mean arterial blood pressure. The values are the mean ± S.E.M. of 5 rats. [a] $P < 0.05$ as compared to the basal MABP. [b] $P < 0.05$ as compared to values obtained at the maximal hypertension.

3.7. Effect of the angiotensin receptor antagonist losartan and the endothelin ET_A receptor antagonist FR 139317

The intravenous bolus injection of angiotensin II (10 ng/kg) induced a short-lasting increase in mean arterial blood pressure (50.0 ± 3.1 mm Hg) which was significantly reduced (14.8 ± 0.5 mm Hg, $n = 5$, $P < 0.01$) in animals pretreated with losartan (10 mg/kg i.v.; Table 1). At this dose, losartan reduced ($P < 0.05$) the hypotension without affecting the hypertension induced by *Phoneutria nigriventer* venom ($n = 5$; Table 2).

The bolus injection of endothelin-1 (3 μg/kg, $n = 7$) induced a brief hypotension (37.0 ± 6.0 mm Hg) followed by a sustained hypertension (25.3 ± 2.5 mm Hg). The intravenous administration of FR 139317 (30 mg/kg) did not affect the hypotension (37.0 ± 5.0 mm Hg) but significantly reduced the endothelin-1-induced hypertension (7.7 ± 2.9 mm Hg, $P < 0.01$). FR 139317 (30 mg/kg) had no effect on the blood pressure changes induced by *Phoneutria nigriventer* venom ($n = 7$, Table 1).

3.8. Effect of the ATP-dependent K^+ channel blocker glibenclamide

Pretreatment of the animals with glibenclamide (50 mg/kg i.p., 30 min before) reduced the hypotension induced by the K^+ channel opener cromakalim (10 μg/kg) from 26.9 ± 4.0 mm Hg to 15.0 ± 3.0 mm Hg ($n = 6$; $P < 0.01$) but it had no effect on the hypotension induced by sodium nitroprusside (1 μg/kg, $n = 7$; Fig. 3). At the dose used above glibenclamide significantly reduced the hypotension induced by *Phoneutria nigriventer* venom without affecting the hypertension ($n = 11$; Fig. 3).

3.9. Effect of Ca^{2+} channel blockers

Fig. 4 shows the effect of the Ca^{2+} channel blockers verapamil, diltiazem and nifedipine on the mean arterial blood pressure changes induced by *Phoneutria nigriventer* venom (0.3 mg/kg).

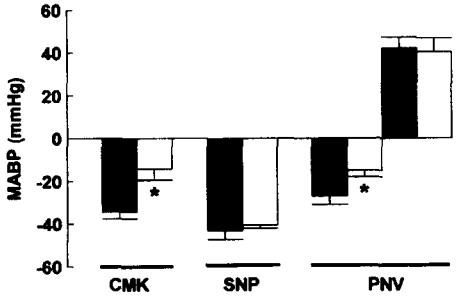

Fig. 3. The effect of glibenclamide (50 mg/kg i.p., 30 min before) on the arterial blood pressure changes induced by intravenous administration of cromakalim (CMK; 10 μg/kg), sodium nitroprusside (SNP; 1 μg/kg) and *Phoneutria nigriventer* venom (PNV; 0.3 mg/kg) in anaesthetised rats. Closed bars represent the control (saline-treated) animals and the open bars represent the glibenclamide-treated animals. Bars represent the means ± S.E.M. * $P < 0.05$ compared to the control values.

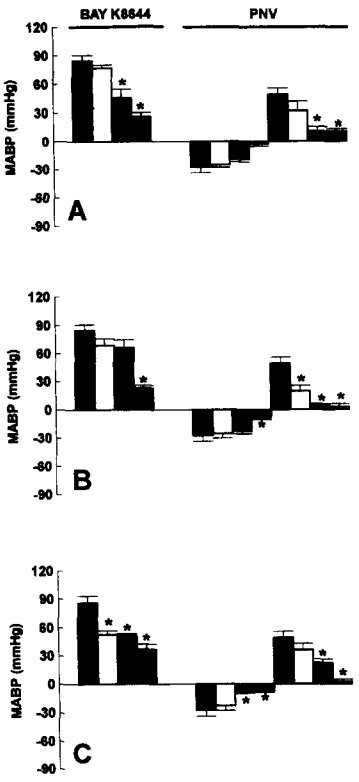

Fig. 4. The effect of verapamil (10–100 μg/kg/min; panel A), diltiazem (40–120 μg/kg/min; panel B) and nifedipine (0.3–10 mg/kg; panel C) on the arterial blood pressure changes induced by *Phoneutria nigriventer* venom (0.3 mg/kg i.v.) in anaesthetised rats. The calcium channel opener BAY K8644 (1 μg/kg i.v.) was injected as controls for the antagonists described above. Closed bars represent the control (saline-treated) animals. The open, striped and cross-hatched bars represent the responses of animals treated with verapamil, diltiazem and nifedipine, respectively. Bars represent the means ± S.E.M. of 5–9 rats. * $P < 0.05$ compared to the control values.

Continuous intravenous infusion of verapamil decreased the basal blood pressure (97.0 ± 4.6 mm Hg before and 82.3 ± 3.2, 81.0 ± 5.0 and 42.0 ± 6.8 mm Hg during 10, 30 and 100 μg/kg/min verapamil infusion, respectively, $P < 0.05$, $n = 5$) and markedly reduced the increase in blood pressure caused by both BAY K8644 and *Phoneutria nigriventer* venom (Fig. 4A). The *Phoneutria nigriventer* venom-induced hypotension was significantly reduced only by the highest dose of verapamil used (Fig. 4A). In addition, the infusion of verapamil (30 μg/kg/min) 5 min following *Phoneutria nigriventer* venom administration (established hypertension) promptly reversed the increased blood pressure ($n = 5$, Table 2).

Intravenous infusion of diltiazem also significantly reduced the basal blood pressure (96.6 ± 4.7 mm Hg before and 68.5 ± 4.9, 70.8 ± 2.6 and 55.0 ± 5.0 mm Hg during 40, 80 and 120 μg/kg/min infusion, respectively, $P < 0.05$, $n = 5$) and markedly reduced the increase in blood pressure caused by both BAY K8644 and *Phoneutria nigriventer* venom (Fig. 4B). Similarly to verapamil, the *Phoneutria nigriventer* venom-induced hypotension was significantly reduced only by the highest dose of diltiazem

used (Fig. 4B). Diltiazem (120 μg/kg/min) also rapidly reversed the hypertension induced by *Phoneutria nigriventer* venom when infused 5 min after venom administration (Table 2).

The effect of nifedipine was evaluated by giving the drug orally 0.5 h before *Phoneutria nigriventer* venom administration. The basal blood pressure was not affected by 0.3 mg/kg (100.6 ± 6.6 and 94.0 ± 4.9 mm Hg for control and treated animals, respectively, $n = 5$) but it was significantly reduced by 1.0 and 10.0 mg/kg (82.5 ± 4.3 and 65.0 ± 4.6 mm Hg, respectively, $P < 0.05$, $n = 5$). At the range of doses used above, nifedipine reduced ($P < 0.05$) both blood pressure changes induced by *Phoneutria nigriventer* venom and the BAY K8644-induced hypertension (Fig. 4C).

Verapamil (30 μg/kg/min), diltiazem (120 μg/kg/min) and nifedipine (10 mg/kg) did not significantly affect the noradrenaline (1 μg/kg)-induced hypertension (58.1 ± 2.9, 64.4 ± 5.6, 55.7 ± 3.5 and 67.0 ± 3.3 mm Hg for control, verapamil-, diltiazem- and nifedipine-treated animals, respectively, $n = 9$).

In the animals treated with verapamil, diltiazem or nifedipine, the increased heart rate induced by *Phoneutria nigriventer* venom was not observed. In addition, the autonomic disturbances described above (salivation, fasciculations and defecation) were not detected and no death was observed even after a second administration of *Phoneutria nigriventer* venom.

4. Discussion

Our results demonstrate that *Phoneutria nigriventer* venom produces a biphasic change in the mean arterial blood pressure of anaesthetised rats which is characterised by a brief hypotension followed by long-lasting hypertension. In guinea-pig auricles, *Phoneutria nigriventer* venom promotes the release of both acetylcholine and noradrenaline from the autonomic nerve endings by a mechanism involving activation of voltage-dependent sodium channels (Vital-Brazil et al., 1988). However, it is unlikely that the initial fall in blood pressure caused by *Phoneutria nigriventer* venom is due to the release of acetylcholine from autonomic nerve endings in the cardiac muscle since the muscarinic receptor antagonist atropine failed to affect this response. We have also ruled out the possibility that *Phoneutria nigriventer* venom increases mean arterial blood pressure by releasing catecholamines since both α- and β-adrenoceptor antagonists (phenoxybenzamine and propranolol, respectively) had no effect on the *Phoneutria nigriventer* venom-induced hypertension.

Kinins (bradykinin and Lys-bradykinin) are known to cause vasodilatation by activating the B_2 receptor subtype (Bhoola et al., 1992). *Phoneutria nigriventer* venom activates the tissue kallikrein-kinin system in rabbit skin (Antunes et al., 1993b; Marangoni et al., 1993a, b; Bento et al., 1995) and rabbit corpus cavernosum (Lopes-Martins et al., 1994) leading to kinin release. The finding that the bradykinin B_2 receptor antagonist Hoe 140 (Wirth et al., 1991) did not affect *Phoneutria nigriventer* venom-induced hypotension excludes the possibility that the venom could be promoting kinin release from the rat plasma. Platelet-activating factor (PAF) is synthesised by different cell types and causes profound hypotension (Braquet et al., 1987). The failure of the PAF receptor antagonist WEB 2086 (Casals-Stenzel et al., 1986) to affect the hypotension induced by *Phoneutria nigriventer* venom indicates that PAF is not involved in this response. *Phoneutria nigriventer* venom stimulates peripheral endings of sensory C-fibers in rat skin, triggering the release of vasoactive neuropeptides such as substance P (Palframan et al., 1995). However, the finding that both tachykinin NK_1 (SR 140333) and NK_2 (SR 48968) receptor antagonists (Emonds-Alt et al., 1992, 1993) failed to affect the fall in blood pressure induced by *Phoneutria nigriventer* venom indicates that this may be restricted to extravascular administration of this venom.

Prostacyclin causes vasodilatation (Moncada et al., 1976) whereas thromboxane A_2 (Hamberg et al., 1975) causes vasoconstriction. Since the cyclooxygenase inhibitor indomethacin affected neither the decrease nor the increase in the blood pressure induced by *Phoneutria nigriventer* venom, we conclude that this venom does not act by stimulating the formation of the above products of arachidonic acid metabolism. The failure of *Phoneutria nigriventer* venom to release either prostacyclin or thromboxane A_2 from guinea pig isolated lungs (Antunes et al., 1993a) further reinforces this conclusion.

The activation of the renin-angiotensin system leads to the release of the potent vasoconstrictor peptide angiotensin II into the circulation thereby causing hypertension. The results showing that the angiotensin II receptor antagonist losartan (Chiu et al., 1990) had no effect on the hypertension induced by *Phoneutria nigriventer* venom indicate that the venom does not stimulate the release of angiotensin II. The mechanism by which losartan significantly reduced the hypotension induced by *Phoneutria nigriventer* venom remains to be elucidated.

Endothelin-1 also induces a biphasic blood pressure response characterized by a brief vasodilatation followed by a prolonged vasoconstriction (Yanagisawa et al., 1988). The vasoconstrictor effect caused by this peptide is mediated mainly by activation of the ET_A receptor subtype (Arai et al., 1990). The failure of the ET_A receptor antagonist FR 139317 (Sogabe et al., 1993) to affect the changes in blood pressure induced by *Phoneutria nigriventer* venom indicates that the venom acts neither by releasing endothelin-1 nor through the presence of endothelin-1-like substances in the venom itself. This contrasts with the occurrence of endothelin-1-like peptides termed sarafotox-

ins in the venom of the snake *Atractaspis engaddensis* (Bdolah et al., 1989). The initial vasodilatation induced by endothelin-1 is due to the release of nitric oxide (De Nucci et al., 1988), a potent vasodilator synthesised by endothelial cells (Furchgott and Zawadzki, 1980). The finding that the nitric oxide synthesis inhibitor L-NAME (Moore et al., 1989) had no effect on the vasodilatation induced by *Phoneutria nigriventer* venom indicates that the venom does not release nitric oxide.

The ATP-dependent K^+ channel blocker glibenclamide (Cook, 1988; Edwards and Weston, 1990) reduced the hypotension induced by both cromakalim and *Phoneutria nigriventer* venom, indicating that the venom activates ATP-dependent K^+ channels. Interestingly, the L-type Ca^{2+} channel antagonists used in this study also inhibited the hypotension induced by *Phoneutria nigriventer* venom. Whether these antagonists also affect K^+ channels is yet to be investigated.

Our results demonstrate that L-type Ca^{2+} channel antagonists such as verapamil (a phenylalkylamine), nifedipine (a dihydropyridine) and diltiazem (a benzothiazepine) greatly reduce *Phoneutria nigriventer* venom-induced hypertension, indicating that the venom opens L-type voltage-dependent Ca^{2+} channels in vivo. Indeed, *Phoneutria nigriventer* toxin PhTX2 increases $[Ca^{2+}]_i$ in rat cortical synaptosomes by a mechanism dependent on the extracellular Ca^{2+} (Romano-Silva et al., 1993). Since L-type Ca^{2+} channels are widely distributed in both the cardiovascular (Spedding and Paoletti, 1992) and the central nervous (Herbette et al., 1994) systems, further studies are necessary to establish the site activated by *Phoneutria nigriventer* venom.

The treatment of *Phoneutria* envenomation in general is symptomatic, attempting to alleviate the intense and radiating pain of the affected member. This is achieved using local anesthetic and/or specific serum therapy (antiarachnid serum) if the pain persists (Lucas, 1988). The severe human envenomation by *Phoneutria nigriventer* venom (especially in children) also causes cardiac perturbations characterized by tachycardia and arrhythmias. Since Ca^{2+} channel blockers have been extensively used in adults and children for the management of cardiovascular disorders (Wagner et al., 1982; Porter et al., 1983; Cho and Pruitt, 1986), our results indicate that it would be worth investigating whether clinical use of commercially available Ca^{2+} channel antagonists could attenuate or even prevent the cardiovascular perturbations and autonomic disturbances elicited by *Phoneutria* envenomation.

Acknowledgements

G.D.N. and S.D.B. thank the Wellcome Trust for support. E.A. thanks Conselho Nacional de Pesquisa (CNPq, Brazil).

References

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

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

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

Arai, S., S. Hori, I. Aramori, H. Ohkubo and S. Nakanishi, 1990, Cloning and expression of a cDNA encoding an endothelin receptor, Nature 348, 730.

Bdolah, A., Z. Wollberg, G. Fleminger and E. Kochva, 1989, SRTX-d, a new native peptide of the endothelin/sarafotoxin family, FEBS Lett. 256, 1.

Bento, A.C., E. Rego, S.R. Pedroso-Mariani, L.C. Mancuso, J.R. Giglio, J.C. Novello, S. Marangoni, I. Caracelli, B. Oliveira, E. Antunes and G. De Nucci, 1995, Isolation of a polypeptide from *Phoneutria nigriventer* venom responsible for the increased vascular permeability in rabbit skin, Toxicon 33, 171.

Bhoola, K.D., C.D. Figueroa and K. Worthy, 1992, Bioregulation of kinins: kallikreins, kininogens and kininases, Pharmacol. Rev. 44, 4.

Braquet, P., T.Y. Shen, L. Touqui and B.B. Vargaftig, 1987, Perspectives in platelet-activating factor research, Pharmacol. Rev. 39, 97.

Brazil, V. and J. Vellard, 1925, Contribuição ao estudo de venenos de aranhas I, Mem. Inst. Butantan 2, 1.

Casals-Stenzel, J.G., G. Muacevic and K.H. Weber, 1986, WEB 2086, a new and specific antagonist of platelet-activating factor (PAF) in vitro and in vivo, Naunyn-Schmied. Arch. Pharmacol. 334, R44.

Chiu, A.T., D.E. McCall, W.A. Price, P.C. Wong, D.J. Carini, J.V. Duncia, R.R. Wexler, S.E. Yoo, A.L. Johnson and P.B.B.W.M. Timmermans, 1990, The non-peptide angiotensin II receptor antagonists: cellular and biochemical pharmacology of losartan, an orally active antihypertensive agent, J. Pharmacol. Exp. Ther. 252, 711.

Cho, C. and A.W. Pruitt, 1986, Therapeutic uses of calcium-channels blocking drugs in the young, Am. J. Dis. Child. 140, 360.

Cook, N.S., 1988, The pharmacology of potassium channels and their therapeutic potential, Trends Pharmacol. Sci. 9, 21.

De Nucci, G., R.J. Gryglewski, T.D. Warner and J.R. Vane, 1988, Receptor-mediated release of endothelium-derived relaxing factor and prostacyclin from bovine aortic endothelial cells is coupled, Proc. Natl. Acad. Sci. USA 85, 2334.

Edwards, G. and A.H. Weston, 1990, Potassium channel openers and vascular smooth muscle relaxation, Pharmacol. Ther. 48, 237.

Emonds-Alt, X., P. Vilain, P. Goulaouic, V. Proietto, D. Van Broeck, C. Advenier, E. Naline, G. Neliat, G. Le Fur and J.C. Brelière, 1992, A potent and selective non-peptide antagonist of the neurokinin A (NK_2) receptor, Life Sci. 50, PL101.

Emonds-Alt, X., J.D. Doutremepuich, M. Healme, G. Neliat, V. Santucci, R. Steinberg, P. Vilain, D. Bichon, J.P. Ducoux, V. Proietto, D. Van Broeck, P. Soubrié, G. Le Fur and J.C. Brelière, 1993, In vitro and in vivo biological activities of SR 140333, a novel potent non-peptide tachykinin NK_1 receptor antagonist, Eur. J. Pharmacol. 250, 403.

Fontana, M.D. and O. Vital-Brazil, 1985, Mode of action of *Phoneutria nigriventer* spider venom at the isolated phrenic nerve-diaphragm of the rat, Braz. J. Med. Biol. Res. 18, 557.

Furchgott, R.F. and J.V. Zawadzki, 1980, The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine, Nature 288, 373.

Hamberg, M., J. Svensson and B. Samuelsson, 1975, Thromboxanes: a new family of biologically active compounds derived from prostaglandin endoperoxides, Proc. Natl. Acad. Sci. USA 172, 2994.

Herbette, L.G., P.E. Mason, K.R. Sweeney, M.W. Trumbore and R.P.

Mason, 1994, Favorable amphiphilicity of nimodipine facilitates its interactions with brain membranes, Neuropharmacology 33, 241.

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

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

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

Marangoni, S., N.C.C. Borges, R.A. Marangoni, E. Antunes, A.C. Vieira, J.C. Novello, G.B. Domont, J.R. Giglio, B. Oliveira and G. De Nucci, 1993b, Biochemical characterization of a vascular smooth muscle contracting polypeptide purified from *Phoneutria nigriventer* (armed spider) venom, Toxicon 31, 377.

Moncada, S., R. Gryglewski, S. Bunting and J.R. Vane, 1976, An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation, Nature 263, 663.

Moore, P.K., A.O. Al-Swayeh, N.W.S. Chong, R.A. Evans and A. Gibson, 1989, L-N^G-Nitro arginine (L-NOARG), a novel L-arginine-reversible inhibitor of endothelium-dependent vasodilation in vitro, Br. J. Pharmacol. 99, 408.

Palframan, R., P. Wilsoncroft, E. Antunes, G. De Nucci and S.D. Brain, 1995, Effect of a tachykinin NK_1 receptor antagonist SR140333 on the oedema formation induced in rat skin by venom from the *Phoneutria nigriventer* spider, Br. J. Pharmacol. 114, 353P.

Porter, C.J., A. Garson and P.C. Gilette, 1983, Verapamil: an effective calcium blocking agent for pediatric patients, Pediatrics 71, 748.

Romano-Silva, M.A., R. Ribeiro-Santos, A.M. Ribeiro, M.V. Gomez, C.R. Diniz, M.N. Cordeiro and M.J. Brammers, 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.

Schenberg, S. and F.A. Pereira-Lima, 1971, *Phoneutria nigriventer* venom. Pharmacology and biochemistry of its components, in: Venomous Animals and their Venoms, Vol. 3, eds. W. Bucherl and E.E. Buckley (Academic Press, New York) p. 279.

Sogabe, K., H. Nirei, M. Shoubo, A. Nomoto, S. Ao, Y. Notsu and T. Ono, 1993, Pharmacological profile of FR 139317, a novel, potent endothelin ET_A receptor antagonist, J. Pharmacol. Exp. Ther. 264, 1040.

Spedding, M. and R. Paoletti, 1992, Classification of calcium channels and the sites of action of drugs modifying channel function, Pharmacol, Rev. 44, 363.

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

Wagner, J.G., A.P. Rochini and J. Vasiliades, 1982, Prediction of steady-state verapamil plasma concentrations in children and adults, Clin. Pharmacol. Ther. 32, 172.

Wirth, K., F.J. Hock, U. Albus, W. Linz, H.G. Alpermann, H. Anagnostopoulos, St. Henke, G. Breipohl, W. Konig, J. Knolle and B.A. Scholkens, 1991, Hoe 140, a new potent and long-acting bradykinin antagonist: in vivo studies, Br. J. Pharmacol. 102, 774.

Yanagisawa, M., H. Kurihara, S. Kimura, Y. Tomobe, M. Kobayashi, Y. Mitsui, Y. Yazaki, K. Goto and T. Masaki, 1988, A novel potent vasoconstrictor peptide produced by vascular endothelial cells, Nature 332, 411.