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Neurohormonal activation in severe scorpion envenomation: Correlation with hemodynamics and circulating toxin

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Abstract

We studied the effects of scorpion (*Androctonus australis hector*) venom on hemodynamics and on the release of catecholamines, neuropeptide Y (NPY), endothelin-1 (ET-1) and atrial natriuretic peptide (ANP) in dog model of severe scorpion envenomation. Nine mongrel anesthetized dogs were submitted to mechanical ventilation through intubation and were administered intravenously purified dried scorpion venom (*Androctonus autstralis*) 0.05 mg/kg. Measurements including pulmonary artery catheter derived parameters, serum toxin levels and humoral variables were performed at baseline (before venom injection) and 5, 15, 30 and 60 min after venom injection. Humoral variables included: serum lactate, epinephrine (EP), norepinephrine (NE), NPY, ET-1 and ANP plasma concentrations. Scorpion venom caused rapid and transient increase of mean arterial pressure (MAP) and PAOP associated with a marked and sustained decline in cardiac output (−55% at 60 min; $P < 0.001$). Hemodynamic changes were associated with a rapid and significant increase of all measured hormones. The highest increase was for NE (28-fold) and EP (25-fold). MAP was closely correlated with NE and less significantly correlated with toxin levels. Similarly, significant correlation was observed between PAPO and ANP plasma levels. These findings support the implication of excessive catecholamines release in hemodynamic disturbances of severe SE and suggest that NPY and ET-1 could be involved in this process. Serum toxin does not appear to consistently contribute to these effects. Through its correlation with PAOP, ANP could be a reliable and useful marker of cardiac dysfunction in SE.

Keywords: Hemodynamics; Peptide hormones; Heart failure; Atrial natriuretic peptide; Endothelins

Introduction

Scorpion envenomation (SE) remains a common life-threatening accident in many tropical and subtropical countries (Goyffon et al., 1982). Scorpion venom induces acute biventricular dysfunction that can progress to death despite aggressive therapy (Abroug et al., 1991, 1995; Elatrous et al., 1999; Gueron et al., 1980, 1992; Nouira et al., 1995). Although the mechanisms accounting for this hemodynamic disturbance are still uncompletely understood, there is now an increasing evidence supporting the pivotal role of a marked release of catecholamines induced by scorpion toxin (Gueron et al., 1980; Zeghal et al., 2000). However, several findings suggest that additional factors could contribute to cardiovascular dysfunction following SE. First, scorpion venom can cause marked alterations in ions channels of excitable cells (Rowan et al., 1992; Yarom and Braun, 1971), and thus can induce by itself a change in myocardial function and vascular tone (Satake et al., 1996; Teixeira et al., 2001). Indeed, some reports have found a correlation between clinical severity of SE and serum level of scorpion toxin (D'Suze et al.,

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2003; Ghalim et al., 2000; Krifi et al., 1998). Second, many of the hemodynamic changes observed in severe SE could be explained by the release of endogenous mediators other than catecholamines (D'Suze et al., 2003; Freire-Maia and de Matos, 1993; Zeghal et al., 2000). We have recently reported the release of number of peptides including endothelin-1 and neuropeptide Y in a dog model of SE (Abroug et al., 2003). The clinical relevance of these findings could be significant and could be targeted to patients victims of scorpion sting should a correlation between this neurohormonal activation and cardiac dysfunction be established in severe SE.

The aim of this prospective animal study conducted in dogs injected with the venom of *Androctonus australis hector*, was:

1. to study the effects of scorpion venom on release profile of plasma catecholamines, NPY, ET-1 and ANP.
2. To assess any correlation between serum toxin levels or humoral changes and hemodynamic parameters.

Materials and methods

Animal preparation. Animals were cared for in accordance with institutional guidelines and the investigation conforms with the *Guide for the Care and Use of Laboratory Animals by the US National Institutes of Health*. Nine mongrel dogs (weight 13 ± 3 kg) were fasted overnight, but allowed water ad libitum. Anesthesia was induced with 5 mg kg^{-1} thiopental and additional doses of 10 mg thiopental were given to maintain depth of anesthesia defined as a slight but present corneal reflex and the absence of signs of pain or discomfort in the studied dogs. The range of total additional doses of thiopental administered for each dog was between 30 and 50 mg. Muscle relaxation was achieved with 0.13 mg kg^{-1} pancuronium bromide given intravenously. The animals were intubated and submitted to volume controlled mechanical ventilation set at a respiratory rate of 20 breaths/min and a tidal volume ranging between 10 and 12 ml/kg adjusted to keep $PaCO_2$ within normal values (40 ± 5 mm Hg). Each dog received 5 ml/kg of intravenous normal saline throughout the preparation. A 16 G arterial catheter was inserted into the femoral artery to measure arterial blood pressure and to withdraw blood for toxin and hormone assays, arterial blood gases, and lactate measurements. A balloon and thermistor-tipped 5-Fr catheter (Arrow international Inc, PA, USA) was inserted and floated through the femoral vein for the measurement of right atrial pressure (RAP), pulmonary artery pressure (PAP), pulmonary artery occluded pressure (PAOP) and cardiac output (CO). The blood temperature determined by means of the pulmonary artery catheter was maintained at normal range during the experiment, using a warming pad.

Measurements. All measurements were taken in the supine position with zero reference level at the mid chest. Cardiac output was measured using standard thermodilution technique with 5 ml of ice-cold saline and expressed as the mean of three measurements. Stroke volume was calculated as CO/HR. Systemic and pulmonary vascular resistances were calculated using standard formulas. Blood gas and lactate measurements were done on automatic analyzer. Blood samples were withdrawn for quantitative determination of scorpion venom toxin and measurement of norepinephrine (NE), epinephrine (EP), neuropeptide Y (NPY), endothelin (ET-1) and atrial natriuretic peptide (ANP). These samples were collected in plastic tubes and then centrifugated and stored at −20 °C until analysis.

Neuropeptide Y, ET-1 and ANP assays were performed in our laboratory according to methods previously described (Cacoub et al., 1993; Eurin et al., 2000; Zongazo et al., 1991). Scorpion toxin was measured using molecular engineering allowing rapid and sensitive immunoassays for the specific detection of the toxin AahI (Aubrey et al., 2001). Briefly, a recombinant plasmid encoding the sc Fv 9C/Strep-tag fusion protein was constructed using monoclonal IgG 9C2 and 2 G3 with *Escherichia coli* strains as cloning and expression hosts. PCR was then performed under standard conditions to reamplify the encoding gene. The recombinant protein was detected rapidly with a dot blot method and then purified by the chromatography of periplasmic preparations extracted from bacterial culture. The antigen-binding activity of the sc Fv 9C2/Strep-tag protein was assessed by radioimmunoassay with various concentrations of unlabeled AahI toxin and compared with the activity of free sc Fv 9C2 and purified IgG 9C2. The last step is the detection and titration of scorpion neurotoxin AahI in animal serum by a sandwich ELISA. The ScFv/Strep-tag conjugate detected AahI at concentrations as low as 2.5 ng/ml.

Samples for NE and E measurements were immediately placed in blood collection tubes with EDTA and reduced glutathione, and then kept on ice until centrifugation at 4 °C. The plasma was separated and stored at −20 °C until analysis. Concentrations of NE and E were determined using a commercial radioenzymatic method (CAT-A-KIT Assay Kit, Amersham Corp, Arlington Heights, IL).

Experimental protocol. After instrumentation, animals were allowed to stabilize for 30 min before any experimental procedure was performed. After baseline measurements of all the variables described above, 0.05 mg kg^{-1} of the purified venom toxic fraction (G50 fraction of the scorpion *Androctonus australis hector*, Institut Pasteur Tunis) was injected in the forearm vein. The venom was obtained from scorpions collected in the multiple oases located in south Tunisia. Venom was water extracted, freeze-dried and stored at −20 °C until use. Its toxic fraction (Aag-FG_{50}) was purified by gel filtration on a sephadex G_{50} column. The lethal potency of the toxic fraction, determined in mice, was about three times

higher than that of crude venom. Hemodynamic measurements and blood sampling for hormonal measurements were repeated at 5, 15, 30 and 60 min after venom injection. At the end of the study, all animals were killed with intravenous potassium chloride.

Statistics. Data are presented as means with standard deviation (SD). Intragroup comparisons were made with Friedman's test for data non-normally distributed. Regression line and correlation coefficients between hemodynamic parameters and plasma toxin levels and humoral data were also calculated. The limit of significance was set at P level <0.05.

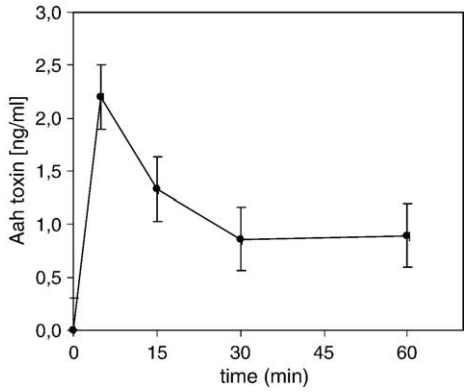

Fig. 1. Time course of serum toxin levels of the scorpion *Androctonus australis hector* (*Aah*) after intravenous injection.

Results

Hemodynamics

Hemodynamic parameters at baseline and their changes over time after venom administration are summarized in Table 1. Mean arterial pressure and PAOP markedly increased after venom injection ($P < 0.001$) while heart rate decreased ($P = 0.04$). The increase in MAP and PAOP occurred rapidly reaching maximum values within 5 min after venom injection (from 124 ± 25 mm Hg to 214 ± 34 mm Hg and from 3 ± 2 mm Hg to 25 ± 7 mm Hg for MAP and PAOP, respectively) with a trend to return towards baseline values thereafter. Venom injection was associated with a rapid and sustained decline in cardiac output until the end of the experiment. Stroke volume remained unchanged during the first 30 min and decreased significantly thereafter. The fall in cardiac output was associated with a sustained increase of systemic vascular resistances ($P < 0.001$) while pulmonary vascular resistances did not change significantly.

Scorpion toxin serum levels

Androctonus australis hector toxin was detected soon after intravenous venom injection. Plasma levels peaked at 5 min and decreased thereafter (Fig. 1); they were significantly correlated to MAP ($r^2 = 0.41$; $P < 0.05$) and inversely correlated to CO ($r^2 = 0.45$; $P < 0.05$).

Hormonal changes

The changes in the concentrations of the studied hormones are summarized in Fig. 2. Plasma levels of all measured hormones raised rapidly and significantly. The most important changes were seen with catecholamines which peaked within 15 min following venom injection (25- and 28-fold increase compared to baseline values, respectively for EP and NE). Similarly, NPY plasma levels increased significantly from the 5th min and continue to increase to reach a level of 7-fold at 60 min. The level of ET-1 increased significantly but slightly from baseline (+38% at 60 min). MAP was positively correlated with EP ($r^2 = 0.46$; $P < 0.05$) and NE ($r^2 = 0.63$; $P < 0.001$). Conversely, no correlation was found between MAP and NPY ($P = 0.50$) nor between MAP and ET-1 ($P = 0.33$). There was a significant correlation between serum toxin concentrations and EP ($r^2 = 0.51$; $P < 0.01$) and NE levels ($r^2 = 0.55$; $P < 0.01$). ANP levels peaked early at the 5th min following venom injection and tended to decrease there-

Table 1
Hemodynamic effects of scorpion venom

	Baseline	5 min	15 min	30 min	60 min	P[a]
MAP (mm Hg)	124 ± 25	214 ± 34*	188 ± 30*	170 ± 30*	134 ± 35	<0.001
HR (bpm)	192 ± 33	142 ± 46*	155 ± 42*	157 ± 40*	172 ± 41	<0.05
CO (L/min)	4.20 ± 1.55	3.33 ± 1.30*	3.27 ± 1.43*	2.98 ± 1.5*	1.92 ± 1.01*	<0.001
SV (ml)	22 ± 7	23 ± 8	23 ± 11	15 ± 6*	11 ± 4*	<0.001
MPAP (mm Hg)	12 ± 3	29 ± 7*	29 ± 9*	23 ± 8*	14 ± 7	<0.001
PAOP (mm Hg)	3 ± 2	25 ± 7*	23 ± 8	19 ± 9*	10 ± 9	<0.001
SVR (dyn s/cm^5)	2551 ± 1074	5007 ± 1867*	5198 ± 2085*	5721 ± 2300*	7078 ± 3015*	<0.01
PVR (dyn s/cm^5)	194 ± 64	112 ± 100	161 ± 86	130 ± 81	253 ± 187	NS

[a] Overall P by ANOVA; NS not significant MAP, mean arterial pressure; HR, heart rate; CO, cardiac output; SV, stroke volume; MPAP, mean pulmonary arterial pressure; PAOP, pulmonary artery occluded pressure; SVR, systemic vascular resistances; PVR, pulmonary vascular resistances.

* $P < 0.05$ vs. baseline.

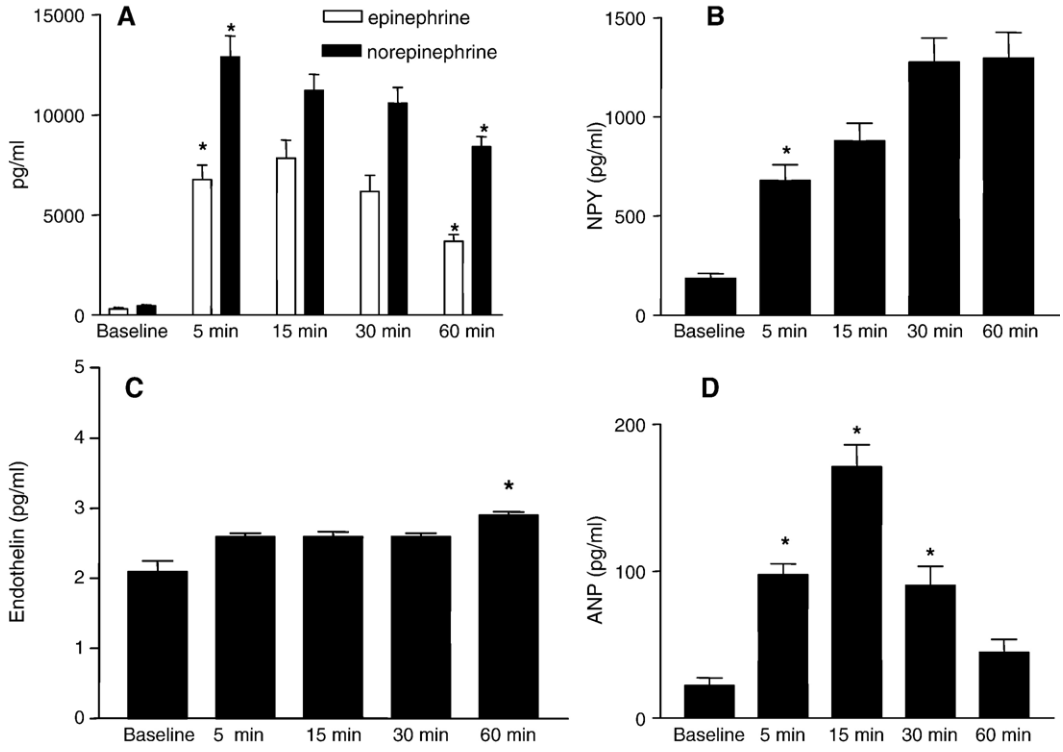

Fig. 2. Time course of plasma concentrations of catecholamines (A), neuropeptide Y (B), endothelin (C) and atrial natriuretic peptide (D) after scorpion venom intravenous injection. All values are given as means ± SD. *$P < 0.05$ vs. baseline.

after. They were significantly correlated with PAOP ($r^2 = 0.55$; $P < 0.01$).

Discussion

In this experimental study, scorpion venom administration caused a marked increase in blood pressure associated with an acute left ventricular failure as demonstrated by a sustained decrease in cardiac output and elevation of PAOP. These hemodynamic alterations were associated with a marked release of circulating catecholamines and significant increase of NPY and ET-1 plasma concentrations. A fair correlation was found between blood pressure and catecholamines as well as scorpion toxin concentrations. Scorpion venom induced a significant increase of ANP plasma levels which were significantly correlated to PAOP.

Clinical studies focusing on hemodynamic consequences of severe scorpion envenomation reported a transient increase in blood pressure followed by a severe cardiogenic shock characterized by a marked decrease in cardiac output and an increase in PAOP usually resulting in pulmonary edema (Abroug et al., 1991, 1995; Elatrous et al., 1999). Injection of the toxin as a bolus into vein does not mimic the clinical envenomation in which absorption from the skin is slower and more erratic. Perhaps subcutaneous injection would be more appropriate. However, all hemodynamic hallmarks of clinical scorpion envenomation are reproduced in our animal model.

The pathogenetic mechanisms underlying cardiovascular dysfunction of SE are not completely understood. However, there is a general agreement to consider that high release of catecholamines from the sympathetic nerves as the major cause of the initial increase in vascular tone and the subsequent hemodynamic failure (Gueron et al., 1980). Yet, the increase of the concentration of catecholamines in the blood or of their metabolites in urine has been shown in only occasional reports (Gueron and Yaron, 1970; Zeghal et al., 2000). Our study is the first one to relate directly arterial pressure changes to circulating catecholamines in SE. The marked increase in SVR is most likely the direct consequences of a great increase in sympathetic nervous activity which produces an extreme degree of peripheral vasoconstriction, leading to acute left ventricular failure reflected by the high PAOP and the fall CO. Besides catecholamines, scorpion venom with its small basic proteins that act on ions channels conductance could per se induce cardiovascular disturbances independently of any neurotransmitter release (Yatani et al., 1988). The ability of scorpion toxins to directly activate the cardiovascular system is well demonstrated on the cardiac and vascular smooth muscle (Satake et al., 1996; Teixeira et al., 2001). In rat aortic rings contracted by phenylephrine, the relaxation induced by isoprenaline was partly inhibited by iberiotoxin (a toxin purified from the scorpion *Buthus tamulus*) (Satake et al., 1996), while on the cardiac level, the venom could induce arrhythmias and contractility defects (Teixeira et al., 2001). Although the question whether these effects are

clinically relevant remains without a clear answer, it is worthy of note that previous studies have emphasized a relation between plasma venom concentrations and the clinical grade of severity (D'Suze et al., 2003; Ghalim et al., 2000; Krifi et al., 1998). Patients with systemic manifestations of envenoming had significantly higher plasma venom concentrations than patients with local signs at the site of the sting. Although MAP is correlated to circulating toxin in the present study, the fact that the same correlation was found with circulating catecholamines makes it difficult to clarify the respective contribution of a direct effect of scorpion venom on the overall hemodynamic profile and a mediated effect through catecholamine release. However, it should be noted that when SVR was at its peak at 60 min, the venom concentration was at its lowest. This mean that circulating toxin is not directly involved in this important vasoconstriction and also explain why delayed administration of antivenom failed to alter any of the SE features (Abroug et al., 1999, 2003). The observation that heart rate was decreased immediately following venom injection raises the possibility that vagal mechanisms may be compromised. It would appear then that SE combined sympathoexcitation and vagal stimulation as well.

The present study also extend our understanding of the process involved in cardiovascular dysfunction by the simultaneous measurement of circulating toxin and number of other vasoactive mediators. Besides catecholamines, we and others hypothesized that scorpion toxin could trigger the release of other mediators involved in the modulation of the circulatory function (D'Suze et al., 2003; Freire-Maia and de Matos, 1993; Zeghal et al., 2000). The potential role of NPY in SE was suggested by the fact that during surgical removal of human pheochromocytoma which is a condition that compares favorably with SE, high levels of plasma NPY were found and positively correlated with systemic vascular resistances (Eurin et al., 2000). The demonstration in our study that plasma levels of NPY increased significantly would support a similar relation in SE. Moreover, the amounts of NPY actually released were far greater than those observed in pheochromocytoma removal (10-fold greater) (Eurin et al., 2000). Although a positive correlation between plasma levels of NPY and MAP was not found in our experiment, it is likely that NPY increase potentiated the effect of catecholamines (Beaulieu and Lambert, 1998; McDermott et al., 1993).

In addition to NPY, ET-1 seems involved in the hemodynamic changes observed in SE. It is interesting to note that the chemical structure of ET-1 is closely related to that of neurotoxins produced by some scorpion species (Williams et al., 1991). The slight increase of ET-1 in our study and the lack of correlation between ET-1 plasma levels and hemodynamics do not exclude its potential role in SE hemodynamic disturbances. Endothelin's propensity for vasoconstriction, combined with its ability to sensitize blood vessels to other vasoactive compounds (Yang et al., 1990), make it a potential candidate in this issue. Indeed, in a rat model of SE using *Buthus occitanus* venom, it was demonstrated that ET-1 antagonist bosentan produced a significant protective effect against venom's pressor action (Zeghal et al., 2000).

Our study also highlights the release of ANP in the particular setting of severe SE and discloses a positive correlation between ANP plasmatic levels and PAOP. Consistent with what is known about the mechanism of release of ANP (de Lemos et al., 2003), this result could have great implications in the overall management of SE since natriuretic peptides are now increasingly used as early biomarkers of cardiac dysfunction (de Lemos et al., 2003). Indeed, one of the most important challenges for clinical practitioners dealing with victims of scorpion sting is to assess accurately and rapidly the need for hospital admission. The lack of objective and readily available data to assess accurately the severity of SE in individual patients is a frequent cause of management failure. The onset of the cardiotoxic effects may be delayed up to few hours but once manifestations appear, it may be too late to prevent further complications (Abroug et al., 1999). Thus, a simple screening test to identify patients at risk of developing severe manifestations after a scorpion sting would be highly desirable in endemic areas with limited care facilities. Biomarkers such as natriuretic peptides assays, which are now commercially available, could provide such information. On the basis of our findings, these emerging diagnostic methods could be an attractive way to guide decision making in SE in a manner that minimizes both patient suffering and unnecessary health care cost. Nonetheless, further work remains before this practice become a routine component of patient care in SE.

In summary, our study lends support to the important role of catecholamines in the pathogenesis of hemodynamic disturbances of SE. Direct action of scorpion toxin on cardiovascular system is likely but appears of minor importance. However, the evidence of the release of newly described peptides in parallel to catecholamines should probably extend the mechanism of SE from the standard explanation of "catecholaminergic pathway" to a more unifying concept called the "peptidic pathway". In a disease where the treatment options are very limited, this neurohormonal activation would probably offer new perspectives in the understanding of SE pathophysiology and the implementation of new pharmacological active agents and new diagnostic tools.

References

Abroug, F., Boujdaria, R., Belghith, M., Nouira, S., Bouchoucha, S., 1991. Cardiac dysfunction and pulmonary edema following scorpion envenomation. Chest 100, 1057–1059.

Abroug, F., Ayari, M., Nouira, S., Gamra, H., Boujdaria, R., Elatrous, S., Ben Farhat, M., Bouchoucha, S., 1995. Assessment of left ventricular function in severe scorpion envenomation: combined hemodynamic and echo-Doppler study. Intensive Care Med. 21, 629–635.

Abroug, F., ElAtrous, S., Nouira, S., Haguiga, H., Touzi, N., Bouchoucha, S., 1999. Serotherapy in scorpion envenomation: a randomised controlled trial. Lancet 354, 906–909.

Abroug, F., Nouira, S., El Atrous, S., Besbes, L., Boukef, R., Boussarsar, M., Marghli, S., Eurin, J., Barthelemy, C., El Ayeb, M., Dellagi, K., Carayon, A., 2003. A canine study of immunotherapy in scorpion envenomation. Intensive Care Med. 29, 2266–2276.

Aubrey, N., Devaux, C., di Luccio, E., Goyffon, M., Rochat, H., Billiald, P., 2001. A recombinant scFv/streptavidin-binding peptide fusion protein for the quantitative determination of the scorpion venom neurotoxin AahI. Biol. Chem. 382, 1621–1628.

Beaulieu, P., Lambert, C., 1998. Peptidic regulation of heart rate and interactions with the autonomic nervous system. Cardiovasc. Res. 37, 578–585.

Cacoub, P., Dorent, R., Nataf, P., Carayon, A., Maistre, G., Piette, J.C., Godeau, P., Cabrol, C., Gandjbakhch, I., 1993. Plasma endothelin and pulmonary pressures in patients with congestive heart failure. Am. Heart J. 126, 1484–1488.

de Lemos, J.A., McGuire, D.K., Drazner, M.H., 2003. B-type natriuretic peptide in cardiovascular disease. Lancet 362, 316–322.

D'Suze, G., Moncada, S., Gonzalez, C., Sevcik, C., Aguilar, V., Alagon, A., 2003. Relationship between plasmatic levels of various cytokines, tumour necrosis factor, enzymes, glucose and venom concentration following Tityus scorpion sting. Toxicon 41, 367–375.

Elatrous, S., Nouira, S., Besbes-Ouanes, L., Boussarsar, M., Boukef, R., Marghli, S., Abroug, F., 1999. Dobutamine in severe scorpion envenomation: effects on standard hemodynamics, right ventricular performance, and tissue oxygenation. Chest 116, 748–753.

Eurin, J., Barthelemy, C., Masson, F., Maistre, G., Soualmia, H., Noe, E., Sarfati, E., Eurin, B., Carayon, A., 2000. Release of neuropeptide Y and hemodynamic changes during surgical removal of human pheochromocytomas. Regul. Pept. 86, 95–102.

Freire-Maia, L., de Matos, I.M., 1993. Heparin or a PAF antagonist (BN-52021) prevents the acute pulmonary edema induced by *Tityus serrulatus* scorpion venom in the rat. Toxicon 31, 1207–1210.

Ghalim, N., El-Hafny, B., Sebti, F., Heikel, J., Lazar, N., Moustanir, R., Benslimane, A., 2000. Scorpion envenomation and serotherapy in Morocco. Am. J. Trop. Med. Hyg. 62, 277–283.

Goyffon, M., Vachon, M., Broglio, N., 1982. Epidemiological and clinical characteristics of the scorpion envenomation in Tunisia. Toxicon 20, 337–344.

Gueron, M., Yaron, R., 1970. Cardiovascular manifestations of severe scorpion sting. Clinicopathologic correlations. Chest 57, 156–162.

Gueron, M., Adolph, R.J., Grupp, I.L., Gabel, M., Grupp, G., Fowler, N.O., 1980. Hemodynamic and myocardial consequences of scorpion venom. Am. J. Cardiol. 45, 979–986.

Gueron, M., Ilia, R., Sofer, S., 1992. The cardiovascular system after scorpion envenomation. A review. J. Toxicol., Clin. Toxicol. 30, 245–258.

Krifi, M.N., Kharrat, H., Zghal, K., Abdouli, M., Abroug, F., Bouchoucha, S., Dellagi, K., El Ayeb, M., 1998. Development of an ELISA for the detection of scorpion venoms in sera of humans envenomed by *Androctonus australis garzonii* (Aag) and *Buthus occitanus tunetanus* (Bot): correlation with clinical severity of envenoming in Tunisia. Toxicon 36, 887–900.

McDermott, B.J., Millar, B.C., Piper, H.M., 1993. Cardiovascular effects of neuropeptide Y: receptor interactions and cellular mechanisms. Cardiovasc. Res. 27, 893–905.

Nouira, S., Abroug, F., Haguiga, H., Jaafoura, M., Boujdaria, R., Bouchoucha, S., 1995. Right ventricular dysfunction following severe scorpion envenomation. Chest 108, 682–687.

Rowan, E.G., Vatanpour, H., Furman, B.L., Harvey, A.L., Tanira, M.O., Gopalakrishnakone, P., 1992. The effects of Indian red scorpion Buthus tamulus venom in vivo and in vitro. Toxicon 30, 1157–1164.

Satake, N., Shibata, M., Shibata, S., 1996. The inhibitory effects of iberiotoxin and 4-aminopyridine on the relaxation induced by beta 1- and beta 2-adrenoceptor activation in rat aortic rings. Br. J. Pharmacol. 119, 505–510.

Teixeira, A.L., Fontoura, B.F., Freire-Maia, L., Machado, C.R., Camargos, E.R., Teixeira, M.M., 2001. Evidence for a direct action of *Tityus serrulatus* scorpion venom on the cardiac muscle. Toxicon 39, 703–709.

Williams Jr., D.L., Jones, K.L., Pettibone, D.J., Lis, E.V., Clineschmidt, B.V., 1991. Sarafotoxin S6c: an agonist which distinguishes between endothelin receptor subtypes. Biochem. Biophys. Res. Commun. 175, 556–561.

Yang, Z.H., Richard, V., von Segesser, L., Bauer, E., Stulz, P., Turina, M., Luscher, T.F., 1990. Threshold concentrations of endothelin-1 potentiate contractions to norepinephrine and serotonin in human arteries. A new mechanism of vasospasm? Circulation 82, 188–195.

Yarom, R., Braun, K., 1971. Electron microscopic studies of the myocardial changes produced by scorpion venom injections in dogs. Lab. Invest. 24, 21–30.

Yatani, A., Kirsch, G.E., Possani, L.D., Brown, A.M., 1988. Effects of New World scorpion toxins on single-channel and whole cell cardiac sodium currents. Am. J. Physiol. 254, H443–H451.

Zeghal, K., Sahnoun, Z., Guinot, M., Richer, C., Giudicelli, J.F., 2000. Characterization and mechanisms of the cardiovascular and haemodynamic alterations induced by scorpion venom in rats. Fundam. Clin. Pharmacol. 14, 351–361.

Zongazo, M.A., Carayon, A., Masson, F., Maistre, G., Noe, E., Eurin, J., Barthelemy, C., Komajda, M., Legrand, J.C., 1991. Effects of arginine vasopressin and extracellular osmolarity on atrial natriuretic peptide release by superfused rat atria. Eur. J. Pharmacol. 209, 45–55.