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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