
Cardiodepressant and neurologic actions of *Solenopsis invicta* (imported fire ant) venom alkaloids

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Background: We hypothesized that the alkaloid compounds that are the majority components of fire ant (*Solenopsis invicta*) venom are capable of producing cardiovascular and central nervous system toxic effects in mammals.

Objective: To evaluate toxic effects of synthetic *S invicta* alkaloids in rodent models.

Methods: Cardiovascular effects of intravenous injection of the racemic (±)-*cis*- and *trans*-isomers of 2-methyl-6-*n*-undecylpiperidine (isosolenopsin A and solenopsin A, respectively) were evaluated in anesthetized, gallamine-paralyzed rats who had received artificial ventilation and in isolated, perfused rat hearts.

Results: (±)-Solenopsin A dose dependently (3–30 mg/kg [10 to 104 μmol/kg]) depressed cardiovascular function. Maximal percent changes following injection of 30 mg/kg were $-42.96\% \pm 5.8\%$ for blood pressure, $-29.13\% \pm 3.6\%$ for heart rate, and $-43.5\% \pm 9.2\%$ for left ventricular contractility (dP/dt). (±)-Isosolenopsin A (3–15 mg/kg [10 to 52 μmol/kg]) produced responses similar to those seen with the corresponding doses of solenopsin A. In conscious, spontaneously breathing rats, solenopsin A (30 mg/kg intravenously) caused seizures, respiratory arrest, and death. Infusion of working, isolated, perfused hearts with solenopsin A reduced contractile function (dP/dt) at 10 μM and caused cardiac arrest at 100 μM.

Conclusions: Two alkaloid components of imported fire ant venom possess robust cardiorespiratory depressant activity and elicit seizures in the rat. Such effects identify these alkaloids as toxic compounds in biological systems and may explain the cardiorespiratory failure noted in some individuals who experience massive fire ant stings.

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INTRODUCTION

Although the properties of the protein components of imported fire ant (*Solenopsis invicta*) venom have been extensively characterized,^{1–3} much less attention has been focused on the alkaloid components that comprise approximately 95% of the venom by volume.⁴ These components have been classified as solenopsins,⁵ isosolenopsins,⁶ or dehydrosolenopsins,⁵ depending on the enantiomeric configuration and alkyl or alkenyl carbon chain in any individual compound. All major identified venom alkaloids have been synthesized.^{5,6}

Continued expansion of the habitat and density of *S invicta* has had an increasingly deleterious impact on human health. Approximately 50% of individuals living in fire ant endemic areas will experience stings within each year, with a variety of resultant medical consequences.⁷ Immediately after stings, all individuals experience intense local burning and a wheal-and-flare reaction. Local allergic reactions and anaphylactic

reactions occur in response to the venom proteins on the basis of venom specific IgE-mediated mast cell degranulation.⁷ The alkaloid components of the venom cause local skin necrosis and are thought to result in the dermal pustule that appears at the sting site 24 to 48 hours after the sting.⁸ Neurologic complications, including seizures and mental status changes, have been reported after fire ant stings, but their origin is unknown.^{9,10}

Massive fire ant sting attacks on humans have occurred both outdoors and indoors, including those of 6 nursing home residents we have recently described.¹¹ The term *massive sting attacks* is used to define case reports in which patients are described to be found, for example, “covered with fire ants”¹¹ or when reports document 500 or more stings per patient. Among those elderly individuals with preexisting cardiopulmonary disease who have been documented to experience such attacks, cardiorespiratory failure has been frequent and most have died within 48 hours.^{10–16} Since only one such massive sting attack has been associated with anaphylaxis,¹¹ adverse effects in most such individuals appear unrelated to allergy. Massive sting attacks are often treated as allergic reactions with antihistamines and corticosteroids even without evidence of allergic reactions. We have suggested that the high mortality rate in these individuals may reflect a combination of direct toxic effects of fire ant venom and adverse effects of corticosteroid and antihistamine ther-

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apy.¹¹ If so, treatment of elderly individuals, in whom the cardiovascular system may already be compromised, with corticosteroids and antihistamines may be inappropriate and could worsen rather than improve their chances for recovery.^{11,15,17} Therefore, additional information is needed on the putative toxic properties of *S invicta* venom and in particular the alkaloid components.

Venom alkaloids include at least 5 compounds, 3 (2R,6R)-*trans*-2-methyl-6-alkylpiperidines, termed *solenopsins*, and 2 (2R,6R)-unsaturated *trans*-2-methyl-6-alkenylpiperidines, termed *dehydrosolenopsins*. Alkyl chains consist of 11 carbons (C11, solenopsin A), 13 carbons (C13, solenopsin B), and 15 carbons (C15, solenopsin C). Dehydrosolenopsin B is a C13 compound with a double bond between C4 and C5 of the chain, whereas dehydrosolenopsin C is a C15 compound with the double bond between C6 and C7. Smaller amounts of the (2R,6S)-*cis*-isomers (referred to by the prefix iso-; eg, isosolenopsin A) of several of these alkaloids are also present in fire ant venom.^{18,19} Figure 1 depicts the structure of (2R,6S)-*cis*-2-methyl-6-*n*-undecylpiperidine (isosolenopsin A) as a reference. Among fire ants, workers of *S invicta* produce venom that is the most complex and toxic.²⁰ Studies by Leclercq et al^{19,21} identified the biosynthetic pathways and absolute configurations for fire ant solenopsins.

Whole fire ant venom has antibacterial,²² histamine-releasing,^{23,24} fungicidal, insecticidal, and hemolytic properties.²⁵ The synthetic C15 venom alkaloids inhibit Na⁺-K⁺ and oligomycin-sensitive Mg²⁺ adenosine triphosphatase (ATPase) activities.^{26,27} The alkaloid derivatives block mammalian neuromuscular transmission postsynaptically through a decrease in the sensitivity of the end-plate membrane to acetylcholine.²⁸ The alkaloid components of the venom also cause activation of platelets and neutrophils through a rise in intracellular calcium.²⁹ All of these properties appear to be of advantage to the fire ant in attacks on arthropod, avian, and small mammal food sources in the wild.

The present study was initiated to evaluate potential mammalian toxic effects to the racemic form of 2 synthetic venom

alkaloids, *cis*- and *trans*-2-methyl-6-*n*-undecylpiperidine. Both of these isomers, (2R,6S)-*cis* and (2R,6R)-*trans*-, are found naturally in *S invicta* venom.^{18–21} The results indicate that these components of *S invicta* venom have the capacity to exert adverse cardiorespiratory and neurologic effects in small mammals. Adverse cardiorespiratory events are consistent with cardiorespiratory compromise that has been documented in massively stung humans.

METHODS

Animal Care

Male (275–325 g) and female (300–350 g) Sprague-Dawley rats were purchased from Harlan Sprague Dawley Inc (Indianapolis, IN) and housed in plastic group shoe box cages in an Association for Assessment and Accreditation of Laboratory Animal Care–approved laboratory animal facility. Animals were housed under a 12-hour light-dark cycle with food and water ad libitum. All animal use protocols were approved by the University of Mississippi Medical Center Institutional Animal Care and Use Committee.

Synthesis of (±)-Solenopsin A and (±)-Isosolenopsin A

The 2-methyl-6-(1-undecenyl)-pyridine ring was prepared from 6-methyl-2-pyridinecarboxaldehyde and decyltriphenylphosphonium bromide via the Wittig reaction.²⁹ Catalytic hydrogenation of 2-methyl-6-(1-undecenyl)-pyridine in the presence of palladium on carbon yielded 2-methyl-6-undecylpyridine. This compound was reduced with sodium in anhydrous ethanol to give a mixture of (±)-solenopsin A and (±)-isosolenopsin A. These 2 synthetic alkaloids were then separated by column chromatography on alumina and crystallized as hydrochloride salts via treatment with hydrochloric acid. Racemic (±)-solenopsin A hydrochloride and racemic (±)-isosolenopsin A hydrochloride have physical (molecular weight, 289.5) and spectral data identical to those previously reported for these 2 synthetic alkaloids.

Alkaloid-Induced Changes in Cardiovascular Function in the Anesthetized Animal

Male rats were anesthetized with 2% to 4% isoflurane in medical grade oxygen. Polyethylene arterial (PE-50) and venous (PE-10) catheters and a tracheal cannula (PE-240) were surgically implanted to permit monitoring of mean arterial blood pressure (MAP) and heart rate (HR), intravenous drug administration, and maintenance of respiration by means of a Harvard rodent respirator, respectively. A catheter (PE-50) was passed via the right carotid artery into the left cardiac ventricle for measurement of left ventricular contractility (dP/dt) as an index of inotropy. Responses were monitored with PowerLab/16 SP data acquisition system using Chart v4.0 for Windows recording software (ADInstruments, Colorado Springs, CO). Immediately following implantation of the tracheal cannula, gallamine triethiodide (10 mg/kg intravenously; Sigma Chemical Co, St Louis, MO) was administered to induce respiratory paralysis. The animal was allowed a period of 15 minutes to achieve a stable baseline of

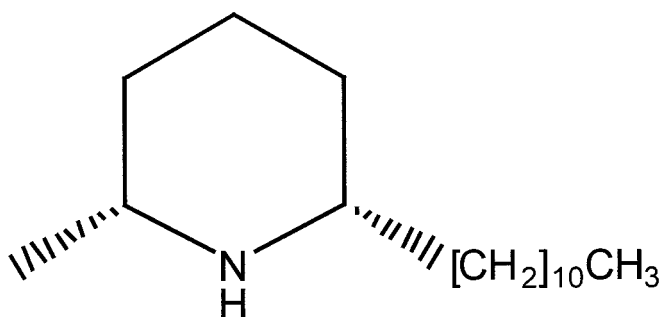


Figure 1. Chemical structure of *cis*-2-methyl-6-undecylpiperidine (isosolenopsin A) found naturally in *Solenopsis invicta* venom. The absolute configuration is (2R,6S)-methyl-6-*n*-undecylpiperidine. In contrast, solenopsin A (*trans*-2-methyl-6-undecylpiperidine) has the configuration (2R,6R)-methyl-6-*n*-undecylpiperidine.

cardiovascular function. Once stable, each animal was administered a volume-matched control (5% β -cyclodextrin in ultrapure water) to the corresponding dose of alkaloid. Maximal changes in HR, MAP, and dP/dt were then recorded. Following control administration, each animal was allowed a period of 1 hour to stabilize. After stabilization, each animal was injected with either ($\pm$)-solenopsin A (3, 15, or 30 mg/kg [10, 52, or 104 μ mol/kg] intravenously) or ($\pm$)-isosolenopsin A (3 or 15 mg/kg [10 or 52 μ mol/kg] intravenously), and maximal changes in HR, MAP, and dP/dt were recorded. Volumes for injection differed by dose due to the low aqueous solubility of the alkaloids. The volumes administered were 3 mL/kg for solenopsin A (30 mg/kg), 2 mL/kg for solenopsin A and isosolenopsin A (15 mg/kg), and 1 mL/kg for solenopsin A and isosolenopsin A (3 mg/kg). Drugs and vehicle were infused for 2 minutes. Data are expressed as percent change from control values measured immediately before solution administration.

Alkaloid Effects in Conscious Animals

Male rats were anesthetized with 2% to 4% isoflurane in medical grade oxygen. Catheters were inserted into the left femoral artery (PE-50) and femoral vein (PE-10) and then exteriorized between each animal's shoulders. Animals were allowed to recover for a minimum of 3 days before use. Before the beginning of the experiment, the animal was placed in a square, Plexiglas experiment cage for approximately 1 hour for acclimation. During this period the animal was connected to a PowerLab/16 SP data acquisition system to record HR and MAP. After the acclimation period, ($\pm$)-solenopsin A (30 mg/kg intravenously) was administered.

Alkaloid Effects on the Isolated Rat Heart

Female rats were anesthetized with pentobarbital sodium (65 mg/kg intraperitoneally) and heparinized (500 IU/kg intraperitoneally). After opening the chest, the aorta was quickly exposed. The pulmonary artery was cut, and the aorta was immediately cannulated with a 16-gauge stainless needle connected to the perfusion system. Retrograde perfusion of the heart with Krebs-Henseleit solution (37°C) was started immediately at a constant flow rate of 15 mL/min. During the perfusion, the heart was excised midsternally and transferred into a water-jacketed chamber (Radnoti Glass Technology Inc, Monrovia, CA). The heart was allowed to stabilize for 5 to 10 minutes before a latex balloon (3 $\times$ 7 mm, 0.03 mL) was inserted into the left ventricle. The balloon was filled with physiologic saline and connected to a pressure transducer via polyethylene tubing (PE-90). Changes in left ventricular pressure (LVP) and its first derivative (dP/dt) were recorded through a data acquisition card (computer board PCI-DAS1602/16; Measurement Computing Corp, Middleboro, MA) by an IBM-compatible PC with DasyLab 6.0 software (Dasytec, Bedford, NH).

Krebs-Henseleit buffer consisted of 118.5mM NaCl, 4.69mM KCl, 1.18mM KH_2PO_4 , 1.16mM MgSO_4 , 2.52mM CaCl_2 , 25.88mM NaHCO_3 , and 5.5mM glucose. This buffer

was freshly prepared and filtered using a 4.5- μ m pore membrane filter (Millipore, Bedford, MA) before use. The Krebs-Henseleit buffer solution was bubbled with 95% oxygen and 5% carbon dioxide to keep a constant pH 7.4 in the perfusion system and was prewarmed to 37°C by a water-jacketed glass chamber with heating coil. The buffer solution flowed through a water-jacketed bubble trap (2-mL volume) immediately before reaching the heart. An outlet tube with adjustable height was connected to the bubble trap to maintain the coronary artery perfusion pressure constant at 80 cm H_2O . During drug treatment, the heart was perfused at a constant pressure with Krebs-Henseleit buffer solution mixed with fixed concentrations of agents.

RESULTS

Effect of ($\pm$)-Solenopsin A and ($\pm$)-Isosolenopsin A on Cardiovascular Function of the Anesthetized Rat

Administration of either solenopsin A (3, 15, or 30 mg/kg intravenously) or isosolenopsin A (3 or 15 mg/kg intravenously) resulted in significant bradycardia in the anesthetized, paralyzed rat (Fig 2). Injection of solenopsin A resulted in maximal reductions in HR of $-6.6\% \pm 0.9\%$, $-19.6\% \pm 3.0\%$, and $-29.13\% \pm 3.6\%$ (mean $\pm$ SEM) at 3, 15, and 30 mg/kg, respectively. Isosolenopsin A administration resulted in maximal reductions in HR of $-6.6\% \pm 1.0\%$ and -18.4%

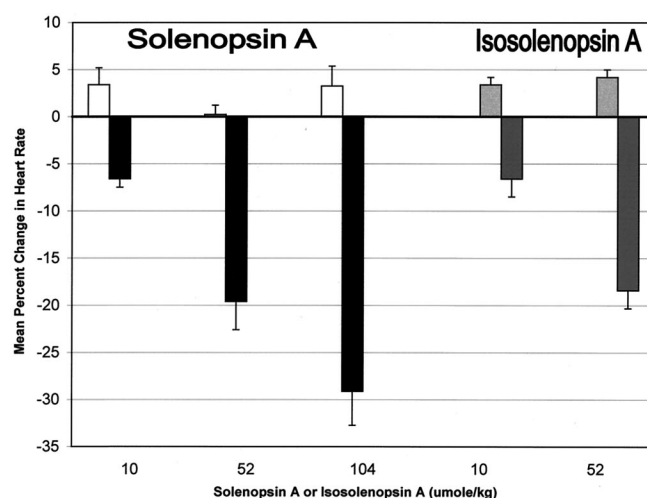


Figure 2. Male rats (275 to 325 g) were anesthetized, catheterized in the left femoral artery and vein, given mechanical ventilation, and paralyzed with gallamine (10 mg/kg intravenously). Each animal was then injected with a volume-matched vehicle (5% cyclodextrin; white and gray bars) to the corresponding dose of either ($\pm$)-solenopsin A or ($\pm$)-isosolenopsin A, and the maximal change in heart rate was recorded. Once the animal stabilized after vehicle injection, either solenopsin A (10, 52, or 104 μ mol/kg intravenously; solid black bars) or isosolenopsin A (10 or 52 μ mol/kg intravenously; cross-hatched bars) was administered, and the maximal change in heart rate was recorded. Each vehicle and corresponding dose of either ($\pm$)-solenopsin A or ($\pm$)-isosolenopsin A represents 4 animals. Values are presented as mean $\pm$ SEM percent change from a control value taken immediately before injection of either vehicle or alkaloid.

$\pm 1.9\%$ (mean $\pm$ SEM) at 3 and 15 mg/kg, respectively. The maximal bradycardia elicited by each alkaloid was achieved within 15 minutes of administration. Administration of 5% cyclodextrin (volume-matched control) resulted in slight tachycardia at the volumes used.

Both solenopsin A (3, 15, or 30 mg/kg intravenously) and isosolenopsin A (3 or 15 mg/kg intravenously) resulted in significant decreases in MAP in the anesthetized, paralyzed rat (Fig 3). Injection of solenopsin A resulted in maximal decreases in MAP of $-17.6\% \pm 1.5\%$, $-38.1\% \pm 3.6\%$, and $-42.96\% \pm 5.8\%$ (mean $\pm$ SEM) at 3, 15, and 30 mg/kg, respectively. Isosolenopsin A administration resulted in maximal decreases in MAP of $-18.4\% \pm 2.4\%$ and $-38.7\% \pm 1.0\%$ (mean $\pm$ SEM) at 3 and 15 mg/kg, respectively. Administration of 5% cyclodextrin (volume-matched control) resulted in an increase of MAP at the volumes used. The maximal decrease in MAP elicited by each alkaloid was achieved within 15 minutes of administration.

The dP/dt was decreased by administration of either solenopsin A (3, 15, or 30 mg/kg intravenously) or isosolenopsin A (3 or 15 mg/kg intravenously). Injection of solenopsin A resulted in maximal decreases in dP/dt of $-16.8\% \pm 0.8\%$, $-24.6\% \pm 5.9\%$, and $-43.5\% \pm 9.2\%$ (mean $\pm$ SEM) at 3, 15, and 30 mg/kg, respectively (Fig 4). Administration of isosolenopsin A resulted in maximal decreases in dP/dt of

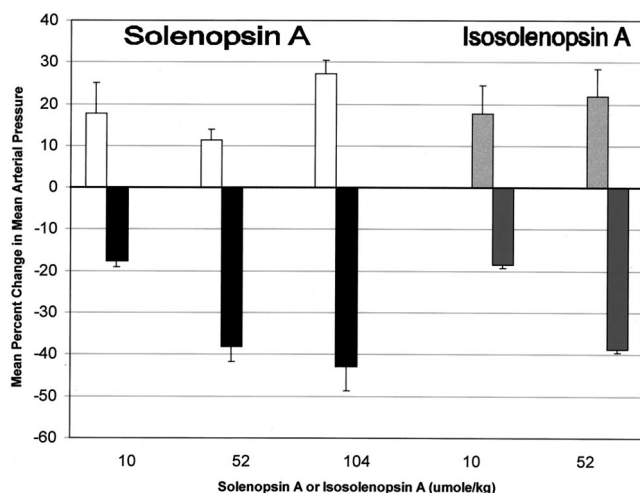


Figure 3. Male rats were anesthetized, catheterized in the left femoral artery and vein, given mechanical ventilation, and paralyzed with gallamine (10 mg/kg intravenously). Each animal was then injected with a volume-matched vehicle (5% cyclodextrin; white and gray bars) to the corresponding dose of either ($\pm$)-solenopsin A or ($\pm$)-isosolenopsin A, and the maximal change in mean arterial blood pressure was recorded. Once the animal stabilized after vehicle injection, either ($\pm$)-solenopsin A (10, 52, or 104 μ mol/kg intravenously; solid black bars) or ($\pm$)-isosolenopsin A (10 or 52 μ mol/kg intravenously; cross-hatched bars) was administered, and the maximal change in blood pressure was recorded. Each vehicle and corresponding dose of either ($\pm$)-solenopsin A or ($\pm$)-isosolenopsin A represents 4 animals. Values are presented as mean $\pm$ SEM percent change from a control value taken immediately before injection of either vehicle or alkaloid.

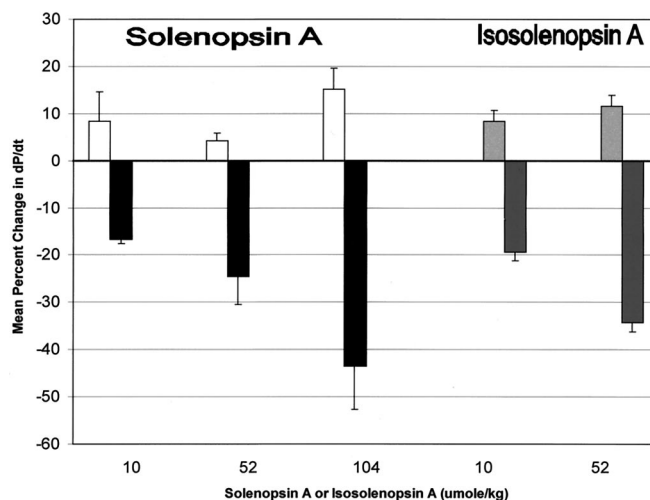


Figure 4. Male rats were anesthetized, catheterized in the left femoral artery and vein, given mechanical ventilation, and paralyzed with gallamine (10 mg/kg intravenously). Each animal was then injected with a volume-matched dose of either ($\pm$)-solenopsin A or ($\pm$)-isosolenopsin A, and the maximal change in left ventricular contractility recorded. Once the animal stabilized after vehicle injection, either ($\pm$)-solenopsin A (10, 52, or 104 μ mol/kg intravenously; solid black bars) or ($\pm$)-isosolenopsin A (10 or 52 μ mol/kg intravenously; cross-hatched bars) was administered, and the maximal change in contractility was recorded. Each vehicle and corresponding dose of either ($\pm$)-solenopsin A or ($\pm$)-isosolenopsin A represents 4 animals. Values are presented as mean $\pm$ SEM percent change from a control value taken immediately before injection of either vehicle or alkaloid.

$-19.3\% \pm 4.2\%$ and $-34.26\% \pm 1.9\%$ (mean $\pm$ SEM) at 3 and 15 mg/kg, respectively. Administration of 5% cyclodextrin (volume-matched control) resulted in an increase of dP/dt at the volumes used. The maximal decrease in dP/dt elicited by each alkaloid was achieved within 15 minutes of administration.

Hemoglobinuria was presumed during postmortem evaluation of the solenopsin A-treated animals by the presence of dark brown and viscous urine in the bladder. This finding was unique to the solenopsin A treatment group. Postmortem gross evaluations of both isosolenopsin A- or 5% cyclodextrin-treated animals were performed. No obvious macroscopic infarcts, discolorations, or hemorrhages were noted on thoracotomy and laparotomy.

Effect of ($\pm$)-Solenopsin A on the Conscious Rat

Administration of solenopsin A (30 mg/kg intravenously; $n = 3$) proved to be lethal in the conscious rat. Bradycardia developed immediately following injection of solenopsin A, with HR decreases of up to 100/min. This was accompanied by an increase in MAP of approximately 25 to 30 mm Hg before onset of seizure activity (data not shown). Within 10 minutes of the injection, all animals experienced tonic-clonic seizures and lethal respiratory arrest. Postmortem evaluation of the animals' bladder revealed the presence of dark brown urine presumptive of hematuria.

Effect of ($\pm$)-Solenopsin A on the Isolated Rat Heart

The effect of solenopsin A on the isolated rat heart was explored using the Langendorff isolated, working, perfused heart preparation with continuous recording from a heart retroperfused with solenopsin A (Fig 5). Solenopsin at concentrations below $30\mu\text{M}$ did not markedly affect LVP (not shown). A dose of $30\mu\text{M}$ solenopsin A reduced peak LVP by $82\% \pm 9\%$ compared with vehicle control in the same heart ($n = 4$). After administration of $100\mu\text{M}$ solenopsin A, the heart arrested. As shown in Figure 5, even after extensive washing, the heart did not recover from exposure to $100\mu\text{M}$ solenopsin A ($n = 7$). The HR in vehicle control hearts was $232 \pm 18/\text{min}$. A dose of $30\mu\text{M}$ solenopsin A did not significantly change the HR (maximal reductions of $89\% \pm 23\%$ vs control). Atropine did not block the effect of solenopsin A on LVP (maximal reductions of $63\% \pm 9\%$ vs atropine only control in the same heart, $n = 3$). Also, the HR was unaffected, since maximal reductions were $87\% \pm 30\%$ compared with the atropine control in the same heart. The latter data demonstrate that solenopsin A does not stimulate M_2 receptors in the heart.

DISCUSSION

Previous studies of the pharmacologic activity of *Solenopsis* alkaloids provided convincing evidence of dermal toxic effects manifested as pustules at sting sites.⁸ The results of the present investigation provide evidence for systemic, multiorgan toxic effects in mammals from the alkaloid components of *S invicta* venom. Although the potential for direct toxic effects of *S invicta* venom has been discounted in the past, the decimation of certain species of ground nesting birds and

small mammals in endemic areas and deaths after massive fire ant stings in elderly people suggested a need for further investigation of *S invicta* venom toxic effects.^{11,30}

The lethal effects of fire ant envenomation have now been described in fish, birds, and small mammals that serve as food sources for these ants.^{31–33} Fire ant venom induces platelet aggregation and activation *in vitro* that could promote thrombosis and the strokes noted in some individuals after massive stings. Our data are presumptive for hemolytic activity of the *trans* conformation of the C11 isomers of solenopsin A; however, we cannot discount a possibility of urine contaminants other than hemoglobin (eg, myoglobin) that may account for the urine discoloration. Our previous finding of selective inhibition of neuronal nitric oxide synthase by *Solenopsis* alkaloids supports the potential for these alkaloids to alter normal systemic physiologic function.³⁴ The data presented herein demonstrate the induction of cardiorespiratory depression, seizure activity, and death in conscious rats by alkaloid components of fire ant venom.

Both C11 isomers appear equipotent as cardiac depressants, because they cause comparable bradycardia, hypotension, and negative inotropic effects following intravenous injection in the anesthetized rat. Similar negative inotropic responses to the *trans* isomer were reproduced in the isolated, perfused myocardium, confirming direct myocardial toxic effects. Since pretreatment in the isolated heart with atropine had no protective effect, these responses are unlikely to be mediated by agonist actions at muscarinic receptors. This conclusion is bolstered by the efficacy of *Solenopsis* alkaloids in anesthetized, gallamine-treated rats, because gallamine possesses selective M_2 receptor antagonism, in addition to blockade of nicotinic neuromuscular receptors.³⁵ Synthetic C11 isomers inhibit nicotinic receptor-associated ion channel function but do not directly block nicotinic receptors.^{28,36} It is unlikely that alkaloid-associated nicotinic receptor blockade blunts cardiovascular ganglionic neurotransmission and induces cardiovascular depression as the effects of the compounds in isolated hearts argue against this. Alkaloid-rich extracts from *Solenopsis richteri*, solenopsin C, and dehydrosolenopsin C (C15 and C15:1) inhibit Na^+, K^+ -ATPase activity,^{26,27} but such inhibition would be expected to enhance cardiac contractility, as do digitalis glycosides. The demonstrated ability of venom alkaloids to release histamine *in vivo*^{23,24} suggests histamine as a putative mediator of the cardiovascular responses to administration of the synthetic alkaloids. Additional research to further elucidate the mechanism of the cardiovascular depressant actions of venom alkaloids is required.

The seizure activity and respiratory arrests that occurred in conscious, spontaneously breathing rats following intravenous injection of solenopsin A also demonstrate the potential for systemic toxic effects of compounds in this series. The structure of 2-methyl, 6-undecylpiperidine (C11) resembles both the precursors of 2,6-disubstituted alkaloids that are present as toxic secretions from the skin of neotropical frogs³⁶

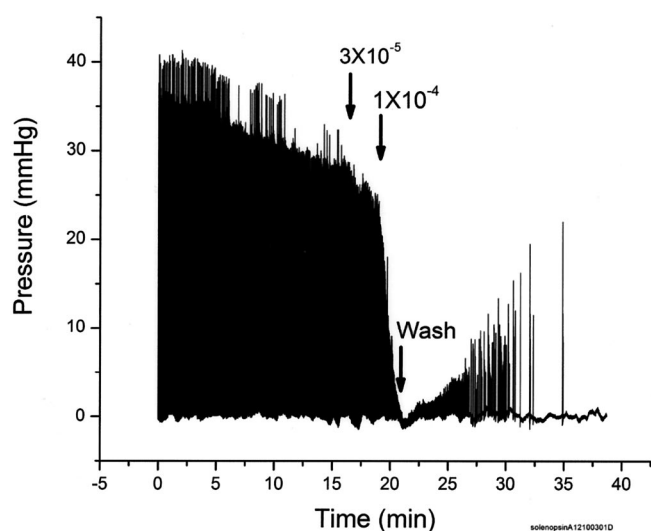


Figure 5. Left ventricular pressure in an isolated, perfused rat heart treated with stepwise increases in ($\pm$)-solenopsin A (3×10^{-5} and 1×10^{-4} M). After administration of 1×10^{-4} M ($\pm$)-solenopsin A into the heart, the heart rapidly stopped beating. The heart did not recover on washing as illustrated in this recording.

and the toxic alkaloids found in poison hemlock, *Conium maculatum*.³⁷

The relative composition of fire ant venom alkaloids varies among species and among castes within a species.^{18,38–40} Both *cis* and *trans* configurations of the solenopsins are present, although the *trans* forms predominate in *S invicta* venom.¹⁸ Approximately 97% of the alkaloid content of venom from workers of *S invicta* consists of *trans*-C13 and C15 compounds, whereas alates contained a much higher percentage (88%) of *cis*- and *trans*-C11 alkaloids.¹⁹ The concentration and composition of the alkaloid components of worker venom change with both age and size of the ants.⁴¹ Likewise, the absolute volume of injectate correlates with size in *S invicta*.^{38,41–43} The mean venom volume delivered in each sting of a *S invicta* worker is 0.66 nL and contains 0.56 μ g of venom alkaloid.⁴² This indicates that, in a healthy 300 g rodent, approximately 1,600 stings would be needed to deliver a threshold systemically toxic dose of 3 mg/kg.

Although one individual recovered from an estimated 10,000 stings without systemic ill effects¹⁶ and a few relatively healthy elderly individuals have experienced massive sting attacks without obvious sequela, 4 (66%) of 6 chronically ill elderly individuals receiving massive fire ant stings while in nursing homes died in less than 1 week of the attack.¹¹ Seizures did not occur in these individuals. One of these individuals had anaphylaxis that responded well to appropriate treatment, but the patient later died in cardiopulmonary failure.¹¹ In most adults who have died after fire ant sting attacks, worsening of preexistent cardiopulmonary disease occurred. Questions that remain to be answered include the relative sensitivity of the human organism to venom alkaloids, the profile of toxic effects of the higher-molecular-weight alkaloid venom components, and the degree of systemic absorption and/or central nervous system penetration of venom alkaloids following cutaneous stings. Without specific data on the relative sensitivity of humans to fire ant venom, the relationship between delivered dose or sting and potential toxic responses cannot be accurately estimated. On the basis of several decades of clinical experience, it is reasonable to assume that humans, in good health, are not especially sensitive to *S invicta* venom toxic effects. However, in the very young or in compromised elderly individuals, aspects of the toxic effects may be exacerbated. In particular, elderly individuals who are already experiencing cardiovascular or respiratory deficits or who are treated aggressively with medications intended to be palliative for insect stings, such as glucocorticoids that promote volume overload or antihistamines that can dry respiratory surfaces and produce sedative effects to further compromise ventilation, are likely to be more sensitive to even subtle cardiovascular and respiratory toxic effects. Many of the elderly individuals whose clinical course has deteriorated following massive fire ant stings have shown a delayed or prolonged decline. This does not necessarily exclude venom toxic effects as a contributing factor. The profound cardiodepressant effects noted *in vivo* and *in vitro* in rat studies suggest that venom alkaloids may irre-

versibly damage cardiac tissue. In cardiologically compromised elderly individuals, the potential to establish a spiral downward of cardiac function over hours or days is a reasonable scenario.

The results of this investigation support the hypothesis that fire ant venom alkaloids can produce respiratory, cardiac, and neurologic toxic effects in mammals. These effects may explain some of the reported clinical sequelae of massive fire ant stings. The increasing spread of the red imported fire ant in the continental United States, Puerto Rico, the Virgin Islands, and Australia⁴⁴ will require further evaluation of both the allergic and toxic properties of *S invicta* venom.

ACKNOWLEDGMENTS

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