Fire Ant Venom Alkaloid, Isosolenopsin A, a Potent and Selective Inhibitor of Neuronal Nitric Oxide Synthase

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Massive, multiple fire ant, *Solenopsis invicta*, stings are often treated aggressively, particularly in the elderly, despite limited evidence of systemic toxicity due to the venom. Over 95% of the *S. invicta* venom is composed of piperidine alkaloid components, whose toxicity, if any, is unknown. To assess a possible pharmacological basis for systemic toxicity, an alkaloid-rich, protein-free methanol extract of the venom from whole ants was assayed for inhibitory activity on the following nitric oxide synthase (NOS) isoforms, rat cerebellar neuronal (nNOS), bovine recombinant endothelial (eNOS), and murine recombinant immunologic (iNOS). Cytosolic NOS activity was determined by measuring the conversion of [^{3}H]arginine to [^{3}H]citrulline in vitro. Rat nNOS activity was inhibited significantly and in a concentration-dependent manner by the alkaloid-rich venom extract. For nNOS, enzyme activity was inhibited by approximately 50% with 0.33 ± 0.06 μg of this venom extract, and over 95% inhibition of the three isoforms, nNOS, eNOS, and iNOS, was found with doses of 60 μg in 60-μl reaction mixture. These results indicate that the alkaloid components of *S. invicta* venom can produce potent inhibition of all three major NOS isoforms. Isosolenopsin A (*cis*-2-methyl-6-undecylpiperidine), a naturally occurring fire ant piperidine alkaloid, was synthesized and tested for inhibitory activity against the three NOS isoforms. Enzyme activities for nNOS and eNOS were over 95% inhibited with 1000 μM of isosolenopsin A, whereas the activity of iNOS was inhibited by only about 20% at the same concentration. The IC_{50} for each of three NOS isoforms was approximately 18 ± 3.9 μM for nNOS, 156 ± 10 μM for eNOS, and >1000 μM for iNOS, respectively. Kinetic studies showed isosolenopsin A inhibition to be noncompetitive with L-arginine ($K_i = 19 \pm 2$ μM). The potency of isosolenopsin A as an inhibitor of nNOS compares favorably with the inhibitory potency of widely used nNOS inhibitors. Inhibition of NOS isoforms by isosolenopsin A and structurally similar compounds may have toxicological significance with respect to adverse reactions to fire ant stings.

Keywords Fire Ant Venom, Isosolenopsin A, Natural Product, Nitric Oxide, nNOS Inhibition

The imported fire ant, *Solenopsis invicta*, has spread throughout the Southeast United States and become a menace to farmland and wildlife (Stafford 1996; Javors et al. 1993). Moreover, infiltration of fire ants into urban areas has greatly increased human contact with them. The chemical and biological aspects of fire ant venom have been extensively studied, but new interest in the toxicity of the venom has arisen with cases of massive fire ant attacks on neonates and in health care facilities (Kemp et al. 2000). The imported fire ant stings multiple times, with each sting containing 0.04 to 0.11 μl of venom (Kemp et al. 2000; deShazo, Butcher, and Banks 1990). The unique venom contains over 95% dialkyl piperidine alkaloids, which have antibacterial (Jouvenaz, Blum, and MacConnell 1972), histamine-releasing (Read, Lind, and Oda 1978; Lind 1982), fungicidal, insecticidal, and hemolytic properties (Javors et al. 1993). The alkaloid components cause the sterile necrotic lesion at the site of envenomation (Rakich et al. 1993). The antigens in the proteins (less than 5%) are probably responsible for any anaphylactic reactions (Kemp et al. 2000; deShazo, Butcher, and Banks 1990). The venom alkaloids of *Solenopsis* species are composed of varying ratios of *cis* and/or *trans* 2-methyl-6-alkyl- or alkenylpiperidines that can be useful taxonomic aids (MacConnell et al. 1976). In *S. invicta* and *S. richteri*, the *trans* forms of alkyl and alkenyl chain piperidines predominate, but in *S. xyloni* and *S. geminata*, *cis* forms are in the majority (Brand et al. 1972; Deslippe and

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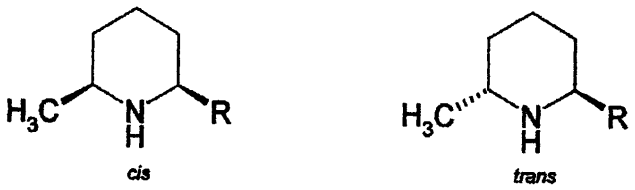

(R = undecyl, tridecyl, pentadecyl, *cis*-4-tridecenyl, or *cis*-6-pentadecenyl)

FIGURE 1
Structures of fire ant venom alkaloids.

Guo 2000; Leclercq et al. 1994, 1996). These alkaloids have been prepared synthetically by MacConnell, Blum, and Fales (1971) (Figure 1).

The venom of *S. richteri* has alkaloid components similar to the alkaloid components of *S. invicta* and inhibits against Na^+-K^+ and oligomycin-sensitive Mg^{2+} ATPase activities (Koch and Desaiah 1975, 1977). The venom of the fire ant has been shown to be toxic to a variety of insects and possesses antibiotic activity toward an assortment of microorganisms (Blum et al. 1958; Jouvenaz, Blum, and MacConnell 1972; Storey et al. 1991). Yeh, Narahashi, and Almon (1975) reported that the alkaloid derivatives block neuromuscular transmission postsynaptically through a decrease in the sensitivity of the end-plate membrane to acetylcholine. The alkaloid components of the venom also cause activation of platelets and neutrophils through a rise in intracellular Ca^{2+} (Javors et al. 1993).

Nitric oxide has important roles as a neurotransmitter, in immune response–induced cytostasis, in the cardiovascular system as a vasodilator, as an inhibitor of platelet aggregation, and in septic shock (Ignarro 2000). Nitric oxide synthase (NOS) catalyzes the production of nitric oxide and L-citrulline through oxidation of L-arginine and by utilizing molecular oxygen and cofactors like NADPH, flavin adenine dinucleotide (FAD), flavin 5′-monophosphate nucleotide (FMN), tetrahydro-L-biopterin (BH_4), and calmodulin (CaM) (Marletta 1993; Alderton, Cooper, and Knowles 2001; Marin and Rodriguez-Martinez 1997). Three NOS isoforms have been identified, two of which, neuronal (nNOS) and endothelial (eNOS), were identified as constitutive forms regulated by Ca^{2+}. The third (iNOS) was identified as a inducible form in macrophages. The overproduction of NO by nNOS may play a role in various neurodegenerative disorders such as Alzheimer's and Parkinson's diseases and other forms of neurotoxicity (Moncada, Palmaer, and Higgs 1991; Kerwin and Heller 1994; Hyman et al. 1992). More potent and selective nNOS inhibitors are under investigation for the treatment of neurotoxic diseases (Sorrenti et al. 2001; Zhang et al. 1997; Alderton, Cooper, and Knowles 2001).

Based on the important roles of nitric oxide in various physiological and inflammatory processes throughout the body and the hypothesis that NOS would be inhibited by *S. invicta* venom alkaloids, extracts of *S. invicta* venom alkaloid were tested against rat cerebellar neuronal, bovine endothelial, and inducible NOS isoforms. Furthermore, isosolenopsin A, a component of *S. invicta* venom alkaloids, was synthesized chemically and tested for inhibitory effects against each of the three isoforms.

MATERIALS AND METHODS

Materials

Three purified NOS isoforms and BH_4 were purchased from Cayman Co. and L-[2,3,4-^{3}H]arginine hydrochloride (40 Ci/mmol) was purchased from New England Nuclear. CaM, $CaCl_2$, Tris-HCl, HEPES, Dowex AG50WX-8 (H^+ form), EDTA, NADPH, FAD disodium salt dihydrate, FMN sodium salt dihydrate were purchased from Sigma-Aldrich. Spin X-centrifuge filters (cellulose acetate; pore size: 0.45 μm) and 7-nitroindazole were purchased from Fisher Scientific.

A total of 1.5 kg of *S. invicta* workers were extracted with hexane as previously described (Vander Meer, Alvarez, and Lofgren 1988). The hexane extract was subjected to silica gel gravity column chromatography to give hexane, chloroform, and methanol fractions. The methanol fraction containing the venom alkaloids was vacuum evaporated leaving ca. 100 ml of residue, then 400 ml of hexane was added and the mixture transferred to a separatory funnel. The mixture was washed three times with 200 ml of 2 N HCl/methanol (1:1). The combined HCl/methanol fractions were made basic with 10% NaOH (ca. 600 ml). The free alkaloids were isolated by extracting the aqueous mixture three times with 200 ml of chloroform. The process starting from the addition of HCl/methanol was repeated two additional times. The chloroform fractions were analyzed by gas chromatography (GC) for venom alkaloids as previously described (Ross et al. 1987). The chloroform extracts were vacuum evaporated to constant weight to yield ca. 4.3 g of venom alkaloids that had the typical GC pattern associated with *S. invicta*. The neat alkaloids were then dissolved in methanol (readily soluble) (maximum concentration was ca. 5% weight/volume).

The racemic isosolenopsin A (*cis*-2-methyl-6-undecylpiperidine) was synthesized by following previously published procedures with little modification (MacConnell et al. 1971; Jefford and Wang 1993; Leclercq et al. 1994). Each observed spectral scan of the product showed the following: IR (AgBr, cm^{-1}): 3350, 2925, 2855, 1463, 1375, 1331 1115, 744; ^{1}H NMR (400 MHz, $CDCl_3$, ppm): 0.87 (t, 3H), 0.98 (m, 2H), 1.05 (d, 3H), 1.24 (br s, 18H), 1.32 (m, 4H), 1.56 (m, 2H), 1.67 (m, 2H), 2.45 (br m, 1H), 2.62 (br m, 1H); and ^{13}C NMR (100 MHz, $CDCl_3$, ppm): 14.18, 22.76, 23.15, 24.96, 26.09, 29.42, 29.68, 29.69, 29.70, 29.74, 29.91, 31.99, 32.31, 34.48, 37.53, 52.57, 57.24. Based on the ^{1}H NMR spectrum, the analyte contained over 95% *cis*-2-methyl-6-undecylpiperidine, with less than 5% trace impurities.

Assay of NOS

NOS activity was measured by monitoring the conversion of [^{3}H]arginine to [^{3}H]citrulline (Bret and Synder 1990). Commercially prepared purified and highly concentrated NOS (nNOS,

602 units/ml, 14.9 mg/ml, and iNOS, 249.7 units/ml, 21.1 mg/ml) were diluted 1:10 in assay buffer before using (Rusche and Marletta 2001; Stevens-Truss and Marletta 1995; Marletta 2001; Hevel, White, and Marletta 2001). Endothelial NOS (32 units/ml, 18.6 mg/ml) was used directly without dilution. Unless otherwise indicated, each tube was incubated at 37°C for 60 minutes. Assay buffer contained 50 mM Tris-HCl (pH = 7.4), 2,3,4-L-[^{3}H]arginine, 2 mM $CaCl_2$, 1 mM NADPH, 10 μM BH_4, 5 μM FAD, 5 μM FMN, and 10 μg/ml CaM. After incubation, the assay reaction was halted by addition of 400 μl of buffer containing 5 mM EDTA and 50 mM HEPES. Equilibrated resin (200 μl, Dowex AG50WX-8 (Na^+ form) was added to the sample reactions and the reaction mixtures were transferred to spin cups and into cup holders. The mixture was passed through columns containing Dowex AG50 resin and the filtrate was collected in a spin cup. The NOS activity was determined by counting the radioactivity (counts/min) in the flow-through (unbound) fraction. Each sample was prepared in duplicate and assays were repeated twice.

Kinetic Measurements

For determinations of K_i, NOS activities were measured by determining the conversion of L-[^{3}H]arginine to L-[^{3}H]citrulline. Reaction mixtures were contained in a final volume of 100 μl, 50 mM Tris-HCl (pH = 7.4), 2 mM $CaCl_2$, 1 mM NADPH, 10 μM BH_4, 5 μM FAD, 5 μM FMN, and 10 μg/ml CaM. Each concentration of L-arginine (33.3 μM, 50 μM, 100 μM) containing the appropriate ratio of L-[^{3}H]arginine (L-arginine: L-[^{3}H]arginine = 1000:1) was prepared to yield serial dilutions of the substrate. The NOS activity was determined by monitoring the conversion of [^{3}H]arginine to [^{3}H]citrulline following a similar method. The K_i value was determined by using Dixon plot analysis.

Statistical Analysis

Data are presented as the mean values ± *SE* ($n = 2\sim4$). The IC_{50} values were calculated from individual plots. One hundred percent NOS activity is defined as the amount of conversion of L-[H^3]arginine to L-[H^3]citrulline by the measurement of CPM in the absence of inhibitor. The ranges of conversion of L-[H^3]arginine to L-[H^3]citrulline by NOS in the absence of inhibitor were 70% to 80% for nNOS, 75% to 85% for iNOS, and 30% to 40% for eNOS respectively. SigmaPlot and SigmaStat were used for statistical plotting and treatment, respectively.

RESULTS

Inhibition of NOS Activity

The addition of fire ant venom alkaloids resulted in a concentration-dependent inhibition of nNOS activity, with approximately 50% inhibition being noted by addition of 0.33 ± 0.06 μg of venom alkaloid (Figure 2). Greater than over 95% inhibition was noted with 25 and 250 μg, respectively. The activity of eNOS and iNOS isoforms was also inhibited 93% ± 5% for eNOS and 92% ± 7% for iNOS following the addition of 1 concentration of 60 μg fire ant venom alkaloids (FAVA).

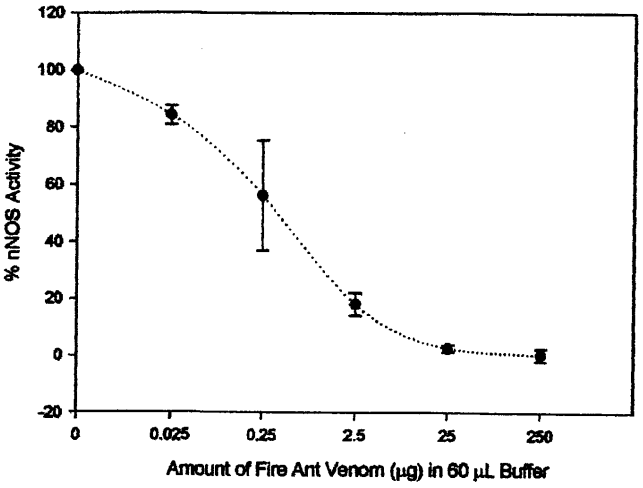

FIGURE 2
Inhibition curve for addition of increasing amounts of FAVA (in μg) to the nNOS assay. Data are expressed as mean ± *SEM*. 100% NOS activity is defined as the amount of conversion of L-arginine to L-citrulline in the absence of inhibitor.

Inhibition of NOS Activity by Isosolenopsin A

The pure isosolenopsin A, *cis*-2-methyl-6-undecylpiperidine, was synthesized and tested for inhibitory activity against all three NOS isoforms. For isosolenopsin A, the enzyme activities for nNOS and eNOS were inhibited by greater than 95% with 1 mM (15.18 μg/60 μl). The iNOS activity was inhibited by only 20% from control at the same concentration. The IC_{50} values for isosolenopsin A were 18 ± 3.9 μM (0.273 ± 0.006 μg/60 μl) for nNOS, 156 ± 10 μM (2.37 ± 0.15 μg/60 μl) for eNOS, and >1000 μM (15.18 μg/60 μl) for iNOS, respectively (Figure 3). The IC_{50} values for 7-nitroindazole (3.5 ± 0.9 μM for nNOS, 12.3 ± 3.0 μM for iNOS, and 7.3 ± 5.0 μM for eNOS), a well-known NOS inhibitor, were obtained in our system to compare with the potency of isosolenopsin A inhibition (Alderton, Cooper, and Knowles 2001).

A kinetic study was conducted to determine the nature of nNOS inhibition by isosolenopsin A. The Dixon plot of enzyme kinetics in the presence of and absence of isosolenopsin A is consistent with a pattern of noncompetitive inhibition with respect to L-arginine for nNOS (Figure 4). The calculated K_i value for isosolenopsin A is 19 ± 2 μM for nNOS.

DISCUSSION

A major finding of this study is the ability of the alkaloid components of *S. invicta* venom to inhibit three NOS isoforms. This suggests mechanisms by which *S. invicta* envenomation might produce both local and systemic pathological reactions,

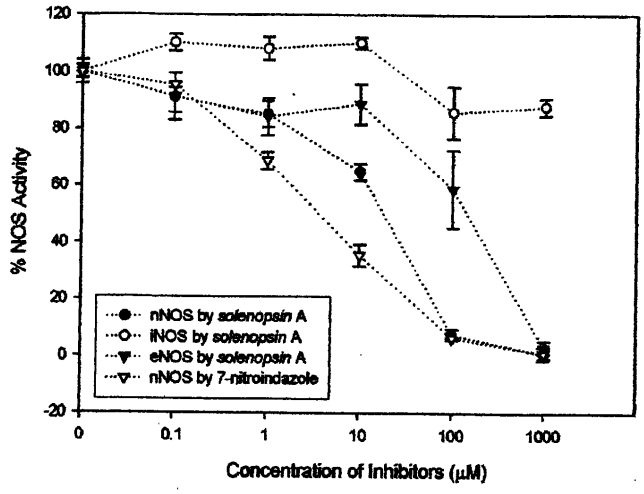

FIGURE 3

Activity inhibition curves for three NOS isoforms (nNOS, iNOS, eNOS) in the presence of isosolenopsin A (*cis*-2-methyl-6-undecylpiperidine) and nNOS activity in the presence of 7-nitroindazole. Data are expressed as mean ± 1 *SEM*. 100% NOS activity is defined as the amount of conversion of L-arginine to L-citrulline in the absence of inhibitor. Standard incubations were performed as described in Materials and Methods using the indicated concentrations of isosolenopsin A and 7-nitroindazole. Calculated IC_{50} values of isosolenopsin A inhibition are 18 ± 3.9 μM for nNOS, 300 μM for eNOS, and >1000 μM for iNOS, respectively. The comparable calculated IC_{50} value of 7-nitroindazole against nNOS is 3.5 ± 0.9 μM.

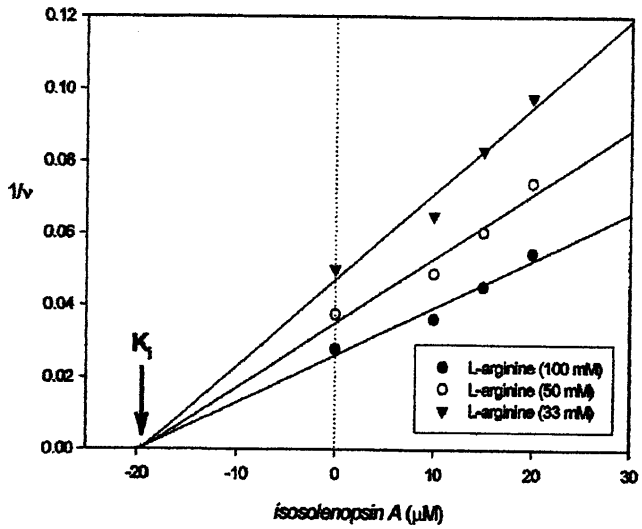

FIGURE 4

Dixon plot of isosolenopsin A inhibition for nNOS. Reaction mixtures were as described in Materials and Methods and the concentration of isosolenopsin A was varied over the range shown (from 0 μM to 20 μM). L-Arginine concentrations were 33 μM ( ), 50 μM (○), and 100 μM (●), respectively.

independently of any of the recognized protein-based hypersensitivity reactions (James et al. 1976; Kemp et al. 2000). In the absence of defined evidence for systemic toxicities of nonprotein venom components, a conservative approach to management of even massive fire ant envenomations has been recommended (deShazo et al. 1999). Previous investigations have shown a variety of biological effects that have been attributed to the alkaloid components of the venom, including antibacterial (Jouvenaz, Blum, and MacConnell 1972; Blum et al. 1958), insecticidal (Blum et al. 1958), platelet-aggregating (Javors et al. 1993), and inhibitory effects on Na^+-K^+ ATPase (Koch and Desaiah 1975, 1977). However, none of these actions seems likely to be responsible for significant systemic toxicity. Yeh, Narahashi, and Almon (1975) observed inhibition of neuromuscular transmission in the frog neuromuscular junction at micromolar concentrations of a synthetic alkaloid (2-methyl-6-undecylpiperidine), but there is no whole animal evidence of paresis or paralysis to extend that observation. Other piperidines with structural similarity to isosolenopsin A, such as coniine, exhibit toxicologically significant acute neuromuscular blockade following oral ingestion. This action has been attributed to nicotinic receptor blockade (Lopez et al. 1999). The transmission blockade observed by Yeh, Narahashi, and Almon (1975) was delayed, requiring 15 to 30 minutes for a maximal effect to become evident. This was suggested to result from poor penetration into muscular tissues and such an action might not affect a sufficient mass of the total neuromuscular junctions to cause a discernable reduction in motor function.

Concern has been expressed about potential neurological impairment following massive envenomation, particularly in a case involving a single newborn (Hardwick et al. 1992). The inhibition of nNOS that has been observed in the present study does suggest a mechanism that could account for neurological complications. The ability of the alkaloid-enriched venom extracts to inhibit two other major NOS isoforms suggests additional toxic potential. Of particular concern is the induction of a hypercoagulable state by platelet activation (Javors et al. 1993) and loss of NO inhibition of platelet aggregation.

A second major finding of this study is that *cis*-2-methyl-6-undecylpiperidine (isosolenopsin A), a synthetic alkaloid from *Solenopsis* species venom, inhibits nNOS selectively. On the basis of IC_{50} values with each isoform, it is apparent that there is a considerable degree of selectivity in favor of nNOS. The potency of inhibition of nNOS by *cis*-2-methyl-6-undecylpiperidine is over 55 times that of iNOS and 8.7-fold that of eNOS. To the best of our knowledge, this *cis*-2-methyl-6-undecylpiperidine is the first synthetic natural product that acts as a selective inhibitor of nNOS. These data compare favorably with the inhibitory potency of widely used nNOS inhibitors, such as 7-nitroindazole, which is not a selective inhibitor for nNOS (Alderton, Cooper, and Knowles 2001; Babbedge et al. 1993). Isosolenopsin A

exerts a noncompetitive inhibition like the imidazole derivatives (Sorrenti et al. 2001), contrary to the competitive NOS inhibitors (Zhang et al. 1997; Desaiah et al. 1999; Frey et al. 1994).

Isosolenopsin A comprises only a trace amount of the venom alkaloids content from *S. invicta*, but is much more prevalent in other *Solenopsis* species (Brand et al. 1972; Deslippe and Guo 2000; Leclercq et al. 1994, 1996). Demonstration that this compound is a potent, selective inhibitor of nNOS, coupled with the finding that alkaloids-enriched venom from *S. invicta* inhibits all major NOS isoforms, strongly suggests that NOS inhibition is a class effect of this alkaloid family. Furthermore, the results indicate that solenopsins other than isosolenopsin A may exert selective inhibitory activity against eNOS and/or iNOS isoforms.

In conclusion, our findings suggest that the inhibition of nNOS by fire ant vernom alkaloids may predispose to neurotoxicity and hypercoagulability after massive stings. Isosolenopsin A may also provide a useful research tool for studying biological responses to selective inhibition of nNOS and would be of interest to researchers working in the area of diseases causing excess production of nitric oxide by the nNOS isoform.

These findings indicate that an alkaloid extract of *S. invicta* venom is a potent inhibitor of nNOS, eNOS, and iNOS isoforms. We propose that the inhibition of nNOS by the venom alkaloids be evaluated as a mechanism whereby *Solenopsis* species venom might cause systemic neurotoxic or other systematic toxic effects in human after massive envenomation.

REFERENCES

Alderton, W. K., C. E. Cooper, and R. G. Knowles. 2001. Nitric oxide synthases: Structure, function and inhibition. *Biochem. J.* 357:593–615.

Babbedge, R. C., P. A. Bland-Ward, S. L. Hart, and P. K. Moore. 1993. Inhibition of rat cerebellar nitric oxide synthase by 7-nitroindazole and related substituted indazoles. *Br. J. Pharmacol.* 110:225–228.

Blum, M. S., J. R. Walker, P. S. Callahan, and A. F. Novak. 1958. Chemical, insecticidal, and antibiotic properties of fire ant venom. *Science* 128:306–307.

Brand, J. M., M. S. Blum, H. M. Fales, and J. G. MacConnell. 1972. Fire ant venoms: Comparative analyses of alkaloidal components. *Toxicon* 10:259–271.

Bret, D. S., and S. H. Synder. 1990. Isolation of nitric oxide synthetase, a calmodulin requiring enzyme. *Proc. Natl. Acad. Sci. U.S.A.* 87:682–685.

Desaiah, D., M. Pande, P. J. S. Vig, J. A. Cameron, and S. F. Ali. 1999. In vitro and vivo inhibition of rat brain nitric oxide synthase activity by phencyclidine. *Int. J. Toxicol.* 18:245–250.

deShazo, R. D., B. T. Butcher, and W. A. Banks. 1990. Reactions to the stings of the imported fire ant. *New Engl. J. Med.* 323:62–466.

deShazo, R. D., D. F. Williams, and E. S. Moak. 1999. Fire ant attacks on residents in health care facilities: A report of two cases. *Ann. Intern. Med.* 131:424–429.

Deslippe, R. J., and Y. J. Guo. 2000. Venom alkaloids of fire ants in relation to worker size and age. *Toxicon* 38:223–232.

Frey, C., K. Narayanan, K. McMillans, L. Spack, S. S. Gross, B. S. Masters, and O. W. Griffith. 1994. L-Thiocitrulline. *J. Biol. Chem.* 42:26083–26091.

Hardwick, W. E., J. A. Royall, B. A. Petitt, and S. J. Tilden. 1992. Near fatal fire ant envenomation of a newborn. *Pediatrics* 90:622–624.

Hevel, J. M., K. A. White, and M. A. Marletta. 2001. Purification of the inducible murine macrophage nitric oxide synthase. *J. Biol. Chem.* 266:22789–22791.

Hyman, B. T., K. Marzloff, J. J. Wenniger, T. M. Dawson, D. S. Bredt, and S. H. Synder. 1992. Relative sparing of nitric oxide synthase-containing neurons in the hippocampal formation in Alzheimer's disease. *Ann. Neurol.* 32:818–820.

Ignarro, L. J. 2000. *Nitric oxide: Biology and pathobiology*. San Diego, California; London: Academic Press.

James, F. K., H. L. Pence, D. P. Driggers, R. L. Jacobs, and D. E. Horton. 1976. Imported fire ant hypersensitivity. *J. All. Clin. Immunol.* 58:110.

Javors, M. A., W. Zhou, W. Maas, S. Han, and R. W. Keenan. 1993. Effects of fire ant venom alkaloids on platelet and neutrophil function. *Life Sci.* 53:1105–1112.

Jeffod, C. W., and J. Wang. 1993. An enantiospecific synthesis of *Solenopsin A*. *Tetrahedron Lett.* 34:2911–2914.

Jouvenaz, D. P., M. S. Blum, and J. G. MacConnell. 1972. Antibacterial activity of venom alkaloids from the imported fire ant, *Solenopsis invicta* buren. *Antimicrob. Ag. Chemother.* 2:291–293.

Kemp, S. K., R. D. deShazo, J. E. Moffitt, D. F. Williams, and W. A. Buhner. 2000. Expanding habitat of the imported fire ant (*Solenopsis invicta*): A public health concern. *J. All. Clin. Immol.* 105:683–690.

Kerwin, J. F., and M. Heller. 1994. The arginine-nitric oxide pathway: A target for new drugs. *Med. Res. Rev.* 14:23–74.

Koch, R. B., and D. Desaiah. 1975. Sensitivity of ATPase activities to fire ant venom and abdomen preparations. *Life Sci.* 17:1315–1320.

Koch, R. B., and D. Desaiah. 1977. Effect of piperidines and fire ant venom on ATPase activities from brain homogenate fractions and characterization of Na^+-K^+ ATPase Inhibition. *Biochem. Pharmacol.* 26:983–985.

Leclercq, S., J. C. Braekman, D. Daloze, J. M. Pasteels, and R. K. Vander Meer. 1996. Biosynthesis of the *Solenopsins*, venom alkaloids of the fire ant. *Naturwissenschuften* 83:222–225.

Leclercq, S., I. Thirionet, F. Broeders, D. Daloze, R. Vander Meer, and J. C. Braekman. 1994. Absolute configuration of the *Solenopsins*, venom alkaloids of the fire ant. *Tetrahedron* 50:8465–8478.

Lind, N. K. 1982. Mechanism of action of fire ant (*Solenopsis*) venoms. I. Lytic release of histamine from mast cells. *Toxicon* 20:831–840.

Lopez, T. A., M. S. Cid, and M. L. Bianchini. 1999. Biochemistry of hemlock (*Conium maculatum* L.) alkaloids and their acute and chronic toxicity in livestock. A review. *Toxicon* 37:841–865.

Marin, J., and M. A. Rodriguez-Martinez. 1997. Role of vascular nitric oxide in physiological and pathological conditions. *Pharmacol. Ther.* 75:111–134.

MacConnell, J. G., M. S. Blum, W. F. Buren, R. N. Williams, and H. M. Fales. 1976. Fire ant venoms: Chemotaxonomic correlations with alkaloidal compositions. *Toxicon* 14:69–78.

MacConnell, J. G., M. S. Blum, and H. M. Fales. 1971. The chemistry of fire ant venom. *Tetrahedron* 26:1129–1139.

Marletta, M. A. 1993. Nitric oxide synthase: Structure and mechanism. *J. Biol. Chem.* 268:12231–12234.

Marletta, M. A. 2001. Approaches toward selective inhibition of nitric oxide synthase. *J. Med. Chem.* 37:1899–1907.

Moncada, S., R. H. Palmaer, and E. A. Higgs. 1991. Nitric oxide: Physiology, pahophysiology, and pharmacology. *Pharmacol. Rev.* 43:109–142.

Rakich, P. M., K. S. Latimer, M. E. Mispagel, and W. L. Steffens. 1993. Clinical and histological characterization of cutaneous reactions to stings of the imported fire ant (*Solenopsis invicta*) in dogs. *Vet. Pathol.* 30:555–559.

Read, G. W., N. K. Lind, and C. S. Oda. 1978. Histamine release by fire ant (*Solenopsis*) venom. *Toxicon* 16:362–367.

Ross, K. G., R. K. Vander Meer, D. J. C. Fletcher, and E. L. Vargo. 1987. Biochemical phenotypic and genetic studies of two introduced fire ants and their hybrid (Hymenoptera: Formicidae). *Evolution* 41:280–293.

Rusche, K. M., and M. A. Marletta. 2001. Reconstitution of pterin-free inducible nitric oxide synthase. *J. Biol. Chem.* 276:421–427.

Sorrenti, V., C. D. Giacomo, L. Salerno, M. A. Siracusa, F. Guerrera, and A. Vanella. 2001. Inhibition of neuronal nitric oxide synthase by *N*-phenacylimidazole. *Nitric Oxide* 5:32–38.

Stafford, C. T. 1996. Hypersensitivity to fire ant venom. *Ann. All. Asthma Immunol.* 77:87–99.

Stevens-Truss, R., and M. A. Marletta. 1995. Interaction of calmodulin with the inducible murine macrophage nitric oxide synthase. *Biochemistry* 34:15638–15645.

Storey, G. K., R. K. Vander Meer, D. G. Boucias, and C. W. McCoy. 1991. Effect of fire ant (*Solenopsis invicta*) venom alkaloids on the *in vitro* germination and development of selected entomogenous fungi. *J. Invertebr. Pathol.* 58:88–95.

Vander Meer, R. K., F. Alvarez, and C. S. Lofgren. 1988. Isolation of the trail recruitment pheromone of *Solenopsis invicta*. *J. Chem. Ecol.* 14:825–838.

Yeh, J. Z., T. Narahashi, and R. R. Almon. 1975. Characterization of neuromuscular blocking action of piperidine derivatives. *J. Pharmacol. Exp. Ther.* 194:373–383.

Zhang, H. Q., W. Fast, M. A. Marletta, P. Martasek, and R. B. Silverman. 1997. Potent and selective inhibition of neuronal nitric oxide synthase by N^{ω}-propyl-L-arginine. *J. Med. Chem.* 40:3869–3870.