Chemical Punch Packed in Venoms Makes Centipedes Excellent Predators*

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Centipedes are excellent predatory arthropods that inject venom to kill or immobilize their prey. Although centipedes have long been known to be venomous, their venoms remain largely unexplored. The chemical components responsible for centipede predation and the functional mechanisms are unknown. Twenty-six neurotoxin-like peptides belonging to ten groups were identified from the centipede venom, Scolopendra subspinipes mutilans L. Koch by peptideomics combined with transcriptome analysis, revealing the diversity of neurotoxins. These neurotoxins each contain two to four intramolecular disulfide bridges, and in most cases the disulfide framework is different from that found in neurotoxins from the venoms of spiders, scorpions, marine cone snails, sea anemones, and snakes (5S animals). Several neurotoxins contain potential insecticidal abilities, and they are found to act on voltage-gated sodium, potassium, and calcium channels, respectively. Although these neurotoxins are functionally similar to the disulfide-rich neurotoxins found in the venoms of 5S animals in that they modulate the activity of voltage-gated ion channels, in almost all cases the primary structures of the centipede venom peptides are unique. This represents an interesting case of convergent evolution in which different venomous animals have evolved different molecular strategies for targeting the same ion channels in prey and predators. Moreover, the high level of biochemical diversity revealed in this study suggests that centipede venoms might be attractive subjects for prospecting and screening for peptide candidates with potential pharmaceutical or agrochemical applications.

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Venomous animals have developed sophisticated chemical strategies to capture prey and defend themselves from predators (1, 2). Most of them secrete venoms containing substances with a wide variety of pharmacological activities to acutely interfere with the physiology of the prey or predator. The most thoroughly studied venomous animals are 5S animals (cone snails, scorpions, sea anemones, snakes, and spiders) (3–12), frogs (13), and insects including ants, bees, and wasps (14). However, there are many other venomous animals including anguimorphs, birds, corals, fishes, jellyfishes, mammals, ticks, and also centipedes. Centipedes have long been known to be venomous, but their venoms have been little studied (15–17).

One of the most obvious similarities shared by most of 5S venoms is that they contain many disulfide-rich peptide neurotoxins that act on ion channels or receptors (18). Spider venoms contain an extreme diversity of small peptide neurotoxins (20–50 amino acids with three to five disulfide bridges) that are targeted to neuronal receptors and ion channels (Ca\(^{2+}\), Na\(^{+}\), K\(^{+}\), and Cl\(^{-}\) channels) (7–9). Scorpion venoms also contain many peptide neurotoxins, including short chain peptides (22–47 amino acids) acting on K\(^{+}\) channels (10) and long chain peptides (58–76 amino acids) acting on Na\(^{+}\) channels (11). Some of them can interact with Ca\(^{2+}\) or Cl\(^{-}\) channels (12). Conus venoms are rich chemical cocktails with diverse range of peptides. Most disulfide-rich conotoxins from Conus venoms are 12–30 residues in length, and they mostly act on ligand-gated or voltage-gated ion channels (4). Several superfamilies have been defined according to differences in their signal sequence and disulfide framework (4). The venoms of Elapidae snakes are a rich source of three-fingered neurotoxins, 60–70-residue polypeptides that act on particular subtypes of voltage-gated ion channels in the brain and at neuromuscular junctions (19). At least two classes of neurotoxins have been identified from sea anemones that act on sodium or potassium channels (20).

Despite their abundance and often painful encounters with humans, little is known about the composition of centipede venom (15–17). Centipede envenomations are capable of inflicting severe symptoms in humans, including myocardial ischemia and infarction, hemoglobinuria and hematuria, hemorrhage, and rhabdomyolysis, itching, fever and chills, general rash, eosinophilic cellulitis, and anaphylaxis. The long list of symptoms and complications induced by centipede envenomations suggests that centipede ven-
oms contain a variety of different components with diverse functions (15–17).

Centipedes are excellent predators. Their prey includes both vertebrates and invertebrates, including bats, rats, amphibians, reptiles, and insects. Centipede venom causes instant rigid paralysis in envenomated houseflies, cockroaches, and crickets, implying that there are neurotoxins in centipede venoms that provide an efficient means of rapidly paralyzing prey (15–17). However, until now, no convincing information has been available about the molecular nature of these neurotoxins. Based on the observed symptoms induced by centipede envenomations and the fast acting manner in which prey is subdued, we hypothesized that centipede venoms contain neurotoxins acting on ion channels and/or receptors that induce rapid paralysis.

In the current work, we attempt to provide the first molecular evidence for the presence of neurotoxins in centipede venoms and to investigate the effect of these neurotoxins on insects and vertebrate ion channels. Assay-guided fractionation was used to purify 10 peptide neurotoxins from the venom of the centipede Scolopendra subspinipes mutilans. Complete amino acid sequences were determined for each of these toxins using a combination of Edman degradation and RACE analysis of a venom gland cDNA library. The cDNA sequences for the complete toxin transcripts indicated that each of these toxins is produced as a larger precursor that is post-translationally processed to yield the mature neurotoxin. The mature toxins ranged in size from 31 to 83 residues, and contained two to four disulfide bonds, which is similar to the mass range observed for neurotoxic peptides in arachnid venoms (21).

MATERIALS AND METHODS

Venom Collection—Adult S. subspinipes mutilans L. Koch (both sexes, n = 3000) were purchased from Jiangsu Province of China. Venom was collected manually by stimulating the venom glands in the first pair forceps of centipedes using a 3 V alternating current as in our previous report (22). The venom were stored at −20 °C until further use. Each milking occurred 1 week after the previous milking.

Neurotoxin Purification—One milliliter of venom was diluted with 3 ml of 0.1 M phosphate buffer, pH 6.0 (PBS) and mixed with 20 μl of proteinase inhibitor mixture (Sigma; P8340-5). The diluted venom (4 ml, total absorbance at 280 nm is 200) was applied to a Sephadex G-50 (Superfine; Amersham Biosciences; 2.6 × 100 cm) gel filtration column equilibrated with 0.1 M PBS. Elution was performed with the same buffer, collecting fractions of 3.0 ml. The absorbance of the eluate was monitored at 280 nm. The effects of eluted fractions on ion channel current were assessed by using patch clamp electrophysiology as described below. The fractions containing interesting activity were pooled (30 ml), lyophilized, resuspended in 2 ml of 0.1 M PBS, and purified further using C18 reverse phase (RP)1 HPLC (Gemini C18 column, 5-μm particle size, 110 Å pore size, 250 × 4.6 mm).

Mass Spectrometric Analysis—Lyophilized HPLC fractions were dissolved in 0.1% (v/v) trifluoroacetic acid/water, and 0.5 μl was spotted onto a MALDI-TOF plate with 0.5 μl of α-cyano-4-hydroxycinnamic acid matrix (10 mg/ml in 60% acetonitrile). The spots were analyzed by an UltraFlex I mass spectrometer (Bruker Daltonics) in a positive ion mode.

Peptide Sequencing—Partial or complete amino acid sequences of purified neurotoxins were determined by Edman degradation using a pulsed liquid phase Pricise® Sequencer, model 491 (Applied Biosystems).

cDNA Synthesis—cDNA was prepared as we described previously (23). Total RNA was extracted from the venom glands of 20 centipedes using TriReZol (Invitrogen), and this was used to prepare cDNA using a SMARTTM PCR cDNA synthesis kit (Clontech). The first strand was synthesized by using the 3’ SMART CDS Primer II A (5’-AACACGGTGATATACAGAGTACT10056N3’-N3’, where N = A, C, G, or T and N, = A, G, or C) and SMART II A oligonucleotide, (5’-AACACGGTGATATACAGAGTACT10056N3’). The 5’ PCR primer II A (5’-AACACGGTGATATACAGAGTACT10056N3’) provided by the kit was used to synthesize the second strand using Advantage polymerase (Clontech).

cDNA Cloning—RACE was used to clone transcripts encoding venom neurotoxins from the venom gland cDNA library (24). Sense direction primers were designed according to the amino acid sequences determined by Edman degradation (see supplemental Table S1 for a list of primers). These primers were used in conjunction with an antisense SMARTTM II A primer II in PCRs to screen for transcripts encoding neurotoxins. PCR was performed using Advantage polymerase (Clontech) using the following conditions: 2 min at 94 °C, followed by 30 cycles of 10 s at 92 °C, 30 s at 50 °C, 40 s at 72 °C. Finally, the PCR products were cloned into pGEM®-T Easy vector (Promega, Madison, WI). DNA sequencing was performed on an ABI PRISM 377 DNA seqencer (Applied Biosystems).

Patch Clamp Recording on Rat Dorsal Root Ganglion (DRG) Neurons—Rat DRG neurons were acutely dissociated and maintained in a short term primary culture according to procedures adapted from Xiao et al. (25). Ca2+, Na+, and K+ currents were recorded from cells using whole cell patch clamp technique using an Axon Multiclamp 700B amplifier (Molecular Devices). The P/4 protocol was used to subtract linear capacitive and leakage currents. The experiment data were acquired and analyzed by using the program Clampfit10.0 (Molecular Devices) and Sigmaplot (Sigma). All of the experimental protocols using animals were approved by the animal care and use committee at Kunming Institute of Zoology, Chinese Academy of Sciences (2011–2012).

Insect Bioassays—Purified neurotoxins were dissolved in insect saline (concentrations in deionized water: 140 mM NaCl, 5 mM KCl, 4 mM NaHCO3, 1 mM MgCl2, 0.75 mM CaCl2, 5 mM HEPES) and injected into American cockroaches (Periplaneta americana), mass 700–900 mg; houseflies (Musca domestica larvae and adults; mass 35–45 mg and 15–25 mg, respectively), and mealworms (Tenebrio molitor larvae; mass 190–210 mg). The median lethal dose (LD50) was determined according to the method described by Tedford et al. (26).

RESULTS

Purification of Neurotoxins from Centipede Venom—Fractionation of venom from the scolopendromorph centipede S. subspinipes mutilans using Sephadex G-50 gel filtration yielded seven protein fractions (Fig. 1A). Fraction V activated voltage-gated Ca2+ (CaV) channels in rat DRGs, whereas fraction VI inhibited K+ currents and tetrodotoxin-sensitive (TTX-S) voltage-gated Na+ (NaV) currents in rat DRGs (data not shown).

Fraction V from the gel filtration separation was subjected to C18 RP-HPLC chromatography as illustrated in Fig. 1B. The peak eluting at 39 min (‘5p-1g’ in Fig. 1B) activated CaV.
currents in rat DRGs. This peak (5p-1g) was fractionated further by C18 RP-HPLC using a shallower acetonitrile gradient (Fig. 1C). The peak eluting at 25.5 min in the chromatogram shown in Fig. 1C (peak 5p-1g-4) activated CaV currents in rat DRGs (as described below), and MALDI-TOF-MS analysis (supplemental Fig. S1E) revealed it was a homogenous peptide with a mass of 8810.4 Da.

Fraction VI from the gel filtration separation was subjected to C18 RP-HPLC chromatography as illustrated in Fig. 1D, resulting in the elution of more than 20 peaks. Peaks 6p-1g, 6p-3g, and 6p-6g were found to inhibit K currents in DRG neurons, peak 6p-4g was found to inhibit TTX-S NaV channel currents, and peak 6p-5g was found to activate CaV channel currents. All of these fractions were purified further using C18 RP-HPLC (Fig. 1, E–I). Peaks 6p-1g-2 (6050.2 Da), 6p-3g-1 (3465.8 Da), 6p-4g-1 (3762.5 Da), 6p-5g-2 (6015.2 Da), and 6p-6g-1 (7989.07 Da) were shown to be homogeneous by MALDI-TOF-MS analysis (supplemental Fig. S1). As described below, 6p-1g-2, 6p-3g-1, and 6p-6g-1 are neurotoxins that act on K+ channels. 6p-4g-1 inhibits TTX-S NaV channels, and 6p-5g-2 activates CaV channels. The other four peptides indicated as PN1, PN4, PN5, and PN9 are also purified, but their functions are unknown. Their partial amino acid sequences were determined by Edman degradation as illustrated in Fig. 8.

**Characterization and Function of Neurotoxins Acting on NaV Channels**

6p-1g-2 (named \(\mu\)-SLPTX-Ssm1a; \(\mu\)-SLPTX-Ssm2a; \(\kappa\)-SLPTX-Ssm3a) was found to inhibit TTX-S NaV channel currents in rat DRGs as illustrated in Fig. 2. NaV currents were elicited by a depolarization to \(+10\) mV from a holding potential of \(-80\) mV. As seen in Fig. 2, 10 nM \(\mu\)-SLPTX-Ssm1a inhibited TTX-S NaV current amplitude by \(60\% \pm 5\%\), whereas the TTX-S NaV current amplitude was depressed by almost 100% in the presence of 10 \(\mu\)M \(\mu\)-SLPTX-Ssm1a (Fig. 2B). Because both TTX-S and TTX-resistant (TTX-R) NaV channels are expressed in small DRG neurons, we added 200 nM TTX to the extracellular solution to dissect out TTX-R NaV currents. The application of 10 \(\mu\)M toxin had virtually no effect on TTX-R NaV channels (Fig. 2C). We conclude based on the concentration-response curve shown in Fig. 2D that \(\mu\)-SLPTX-Ssm1a specifically inhibits TTX-S NaV channels with an IC50 of \(\sim 9\) nM.

The complete amino acid sequence of \(\mu\)-SLPTX-Ssm1a determined by Edman degradation was ADNKFENSLRRE-
IACGQCRDKVKCDPYFYHCG (Fig. 2A), yielding a predicted mass of 3763.2 Da for the fully oxidized form of the peptide. This corresponds closely to the mass of 3762.5 Da determined for the native toxin using MALDI-TOF-MS analysis (supplemental Fig. S1A). This indicates that the four cysteine residues in /H9262-SLPTX-Ssm1a form two disulfide bonds. A BLAST search of the UniProt database revealed that /H9262-SLPTX-Ssm1a is a novel peptide.

Characterization and Function of Neurotoxins Acting on K⁺ Channels—Three peptide neurotoxins (6p-1g-2, κ-SLPTX-Ssm2a; 6p-3g-1, κ-SLPTX-Ssm2a; and 6p-6g-1, κ-SLPTX-Ssm3a) were purified and found to inhibit K⁺ channel currents in DRG neurons. The sequence of κ-SLPTX-Ssm1a (51 residues) was determined to be TDDESNKCAKTKRRENYRVRHCDLGRLRN by Edman degradation, mass spectrometry, and cDNA cloning (Fig. 3A and GenBank™ accession number JQ750704-1). The complete toxin transcript cloned from the venom gland cDNA library revealed that the toxin is transcribed as a larger precursor containing a 23-residue propeptide that is removed during post-translational processing. The C terminus of the propeptide contains a classical dibasic processing site (KR), and like most spider venom propeptides, it is highly acidic (18, 27). Five homologues of κ-SLPTX-Ssm1a (predicted κ-SLPTX-Ssm1b-2f) were identified by cDNA cloning (JQ750705-9).

The molecular mass of κ-SLPTX-Ssm2a calculated from the sequence, assuming that the six Cys residues form three intramolecular disulfide bridges, is 3467.1 Da, which represents a difference of 1.3 Da from the mass (3465.8 Da) determined by MS analysis (supplemental Fig. S1B). We therefore conclude that the six Cys residues in κ-scoloptoxin-Ssm1a form three intramolecular disulfide bridges. A BLAST search of the UniProt database revealed no sequences similar to κ-SLPTX-Ssm1a.

As seen in Fig. 3B, 8 nM κ-SLPTX-Ssm1a inhibited K⁺ current amplitude by 25 ± 5%, but almost 100% of the K⁺ current amplitude was depressed when the toxin concentration was increased to 1 μM. Based on the concentration response shown in Fig. 3C, we conclude that κ-SLPTX-Ssm1a inhibits K⁺ currents in DRG neurons with an IC50 of 44.2 nM.
molecular disulfide bridges. A BLAST search of the UniProt database revealed no sequences similar to H9260-SLPTX-Ssm2a.

As seen in Fig. 4B, 200 nM H9260-SLPTX-Ssm2a inhibited K+ current amplitude in DRG neurons by 45 ± 5%, and the current was inhibited by almost 80% when the toxin concentration was increased to 1 μM. Based on the concentration response shown in Fig. 4C, we conclude that H9260-SLPTX-Ssm2a inhibits K+ currents in DRG neurons with an IC50 of 570 nM.

The amino acid sequence of H9260-SLPTX-Ssm3a (68 residues) was determined to be EVIAIDGLEICSNDQLHVTIYS-IFSPLFDKPKLNYFNCSCPGSIYISDVYPKYFNDIAHIEY-RCKLT (Fig. 5A). The complete toxin transcript (GenBank™ accession number JN646116) cloned from the venom gland cDNA library revealed that the toxin is transcribed as a larger precursor containing a 16-residue signal peptide (MSWM-FYSFIVFTLAIK) that is removed during post-translational processing. MS analysis of the native peptide yielded a mass of 7989.07 Da (supplemental Fig. S1), which matches well with the predicted mass based on the sequence (7989.05 Da), assuming that the four Cys residues from two intramolecular disulfide bridges. 200 nM H9260-SLPTX-Ssm3a inhibited K+ current amplitude in DRG neurons by only 25 ± 5% (n = 5), and these currents were completely abolished by 1 μM H9260-SLPTX-Ssm1a; the estimated IC50 was 44.2 ± 5.7 nM (n = 5).

Characterization and Function of Neurotoxins Acting on CaV Channels—Two peptide neurotoxins (5p-1g-4, H9275-SLPTX-Ssm1a; and 6p-5g-2, H9275-SLPTX-Ssm2a) were purified and found to activate or inhibit CaV channel currents. The amino acid sequence of H9275-SLPTX-Ssm1a (83 residues) was determined to be EECPSLSGGSSNYCSKKETFTSNGNLEQTRRY-CNSVAAPSTACTDLKTGGKLCEYSCNTDGCNSVAGMEPTR-AFYFIALMLA (Fig. 5C). The complete toxin transcript (GenBank™ accession number JN646117) cloned from the venom gland cDNA library revealed that the toxin is transcribed as a larger precursor containing a 45-residue prepro-region that is removed during post-translational processing. SignalP (28) predicts that this region is comprised of a 24-
residue signal peptide (MPSLCIALFGTLTFYTLIPSIHT) and an unusual 22-residue propeptide (LKCVRCDGPMSNYDCKT-TYPAA) that is cationic and contains a noncanonical C-terminal cleavage site (AA). The mature toxin sequence comprises 86 amino acid residues and includes 7 cysteines. It appears that six of these Cys residues form three intramolecular disulfide bridges because MS analysis of the native peptide yielded a mass of 8810.4 Da (supplemental Fig. S1E), which represents a difference of 8.4 Da compared with the predicted molecular mass (8818.8 Da) of linear -SLPTX-Ssm1a. -SLPTX-Ssm1a is an activator for CaV channels in DRG neurons. As illustrated in Fig. 5D, 1 mM -SLPTX-Ssm1a increased CaV currents in DRG neurons by 70%, whereas 10 mM toxin increased CaV currents by 120%.

The amino acid sequence of -SLPTX-Ssm2a (54 residues) was determined to be EESQHDTLNEYRLSCIPKGGECER-MADTCCPGQLGCLGCNPNKENLGKCKCQ (Fig. 6A). The complete toxin transcript (GenBank™ accession number JN646118) cloned from the venom gland cDNA library revealed that the toxin is transcribed as a larger precursor containing a 22-residue signal peptide (MAYIYALIFAIVVCMNTDVIA) that is removed during post-translational processing. MS analysis gave an observed mass of 6014.2 Da for the native peptide (supplemental Fig. S1F), which is 9.6 Da less than the mass of linear -SLPTX-Ssm2a (6023.8 Da) calculated from the amino acid sequence. This indicates that the eight Cys residues in -SLPTX-Ssm2a form four disulfide bonds. One homologue (Predicted -SLPTX-Ssm2b) of -SLPTX-Ssm2a was identified by cDNA cloning (JQ757070; Fig. 6A).

As illustrated in Fig. 6B, -SLPTX-Ssm2a is an inhibitor of CaV channel currents in DRG neurons. 500 nM -SLPTX-Ssm2a inhibited CaV current amplitude by only 45 ± 5%, but 80% of the CaV current was inhibited when the toxin concentration was increased to 1 μM. C, concentration-response curve for inhibition of CaV channel currents by -SLPTX-Ssm2a; the estimated IC50 was 570 ± 126 nM (n = 5).
increased to 2.5 μM. The concentration-response curve shown in Fig. 6C indicates that ω-SLPTX-Ssm2a inhibits CaV channel currents in DRG neurons with an IC₅₀ of about 1590 nm.

A BLAST search of the ArachnoServer and UniProt databases revealed that ω-SLPTX-Ssm2a has similarity to several lycotoxins identified in a venom gland transcriptome of the...
Neurotoxin Variety of Centipede Venoms

Fig. 7. Precursor sequences of four peptide families with unknown functions. The amino acid sequences determined by Edman degradation are underlined. PN, predicted neurotoxin. An asterisk indicates a conserved half-cysteine residue.

araneomorph spider Lycosa singoriensis (29–31). However, as shown in Fig. 6A, even though the disulfide frameworks of the two toxins can be aligned, the N and C termini of the toxins are quite different, and the overall level of sequence identity is only ~41%. Thus, even though the spider and centipede toxins might have a similar three-dimensional fold, it is possible that the lycotoxins have a different target and mode of action to \( \omega \)-SLPTX-Ssm2a.

Four Families of Novel Peptides—Four families of novel peptides including 10 members (JQ757071–80) were identified by proteomics and/or transcriptome analysis as illustrated in Fig. 7. Among them, three families have been purified as illustrated in Fig. 1. These peptides contain novel primary structures and cysteine motifs, but their functions are not known. Further work is necessary to characterize their biological functions.

Isolated Centipede Neurotoxins and Crude Venom Have Potent Insecticidal Activity—We tested the lethal activity of \( \mu \)-SLPTX-Ssm1a, \( \kappa \)-SLPTX-Ssm1a, \( \kappa \)-SLPTX-Ssm2a, \( \kappa \)-SLPTX-Ssm3a, \( \omega \)-SLPTX-Ssm1a, and crude centipede venom against blowflies, mealworms, and cockroaches (Fig. 8). All of the toxins except \( \omega \)-SLPTX-Ssm1a had potent insecticidal activity. 10 min to 2 h following injection of toxins or crude venom, these insects showed signs of neurotoxicity including twitching, paralysis, and body contraction. The neurotoxins and venom dose-dependently exerted their lethal toxicities on each of the tested insects. The LD\(_{50}\) for \( \mu \)-SLPTX-Ssm1a ranged from 67 pmol/g (0.25 \( \mu \)g/g) in adult blowflies to 6300 pmol/g (23.7 \( \mu \)g/g) in adult centipede venom (LD\(_{50}\) of 5 pg/g). It appears that the lethal activity of the crude venom is about 3500–50,000 times higher than that of the purified neurotoxins on a mass basis.

DISCUSSION

Centipede Venom Contains Novel Disulfide-rich Peptide Neurotoxins—Centipedes are important venomous arthropods that frequently cause nonlethal human envenomations. However, information about the composition and function of their venom is scarce. Some studies have focused on the pharmacological properties of crude centipede venom or venom fractions. Extracts of the venom gland from Scolopendra morsitans were found to arrest the heart muscle of toads (32). Some other Scolopendra venoms have been found to induce an increase in the leak current (33), to activate muscarinic receptors, and to increase the basal release of neurotransmitters in the ventral ganglia of crustaceans (34). Centipedes have also been demonstrated to be effective and fast-acting bioinsecticides. For example, Rates et al. (15) demonstrated that the crude venom of a single individual of S. viridicornis nigra is capable of killing 100,000 houseflies. In addition, centipede venom fraction was found to induce an increase in the leak current correlated with a decrease in the membrane resistance on the cockroach giant axon (33). All of these reports imply that there are neurotoxin-like components in centipede venoms.

Centipede Venom Peptides Modulate Vertebrate Voltage-gated Ion Channels—All of the centipede venom peptides we isolated modulate particular types of voltage-gated ion channels in rat DRG neurons: \( \mu \)-SLPTX-Ssm1a is an inhibitor of TTX-S Na\(_{v}\) channels; \( \kappa \)-SLPTX-Ssm1a, -Ssm2a, and -Ssm3a are inhibitors of K\(_{v}\) channels; \( \omega \)-SLPTX-Ssm1a acts on Ca\(_{v}\) channels; and \( \omega \)-SLPTX-Ssm2a inhibits Ca\(_{v}\) channels. Most of these pharmacologies are common in the venoms of SS animals. However, with the exception of \( \omega \)-SLPTX-Ssm2a, the centipede venom peptides have no similarity with any known peptide or protein families from any venomous animal. Hence, these peptides are likely to have unique three-dimensional
folds, and they might provide novel scaffolds for engineering peptides with pharmaceutical or bioinsecticidal activity.

In contrast with the other purified centipede venom peptides, \(\omega\)-SLPTX-Ssm1a has a unique activity, namely activation of vertebrate Ca\(_{\text{v}}\) channels. We are not aware of any other venom peptide that has been demonstrated to activate either vertebrate or invertebrate Ca\(_{\text{v}}\) channels. One might have expected activation of insect Ca\(_{\text{v}}\) channels to be lethal because it would presumably cause massive neurotransmitter release at synapses. However, we found that \(\omega\)-SLPTX-Ssm1a was only weakly insecticidal, perhaps suggesting that it is not a particularly potent activator of invertebrate Ca\(_{\text{v}}\) channels.

\(\omega\)-SLPTX-Ssm2a was the only centipede venom peptide with sequence similarity to toxins from other animal venoms. \(\omega\)-SLPTX-Ssm2a has the same number of cysteine residues and similar intercysteine spacings to several lycotoxins identified in a venom gland transcriptome of the spider \(L.\ singoriensis\). However, the overall level of sequence identity between the toxins is only \(-41\%\), indicating that the spider and centipede toxins might have quite different modes of action even if their three-dimensional structures are similar.

**Fig. 8.** Insecticidal activity of purified scoloptoxins and crude centipede venom. A–D, dose-response curves obtained from injection of purified scoloptoxins into blowfly larvae (A), adult blowflies (B), cockroaches (C), and mealworms (D). E, dose-response curve obtained from injection of crude centipede venom into the same insect species. LD\(_{50}\) values calculated from dose-response curves are given in the figures. ST1, \(\mu\)-SLPTX-Ssm1a; ST2, \(\kappa\)-SLPTX-Ssm1a; ST3, \(\kappa\)-SLPTX-Ssm2a; ST4, \(\kappa\)-SLPTX-Ssm3a; ST5, \(\omega\)-SLPTX-Ssm1a.
**Centipede Venom and Purified Centipede Venom Peptides Are Insecticidal**—Both purified neurotoxins and crude centipede venom were strongly insecticidal (Fig. 8), with crude centipede venom being significantly more potent than any of the purified neurotoxins. Based on the calculated LD$_{50}$ values, 1 $\mu$g of purified neurotoxin could kill 160–1,600 adult blowflies, whereas 1 $\mu$g of crude venom is able to kill 8,000,000 blowflies. Approximately 0.4 mg of crude venom can be obtained by milking a single adult S. subspinosus mutlans centipede over a 2-week period, which is sufficient venom to kill 3.2 $\times$ 10$^6$ adult blowflies. The significant difference in insecticidal ability between purified neurotoxins and crude venom most likely arises from the synergistic effects of various neurotoxins and/or the presence of unidentified toxins in the crude venom that have significantly greater insecticidal activity than any of the neurotoxins isolated in the current study. Nevertheless, our work indicates that centipede venoms contain numerous insecticidal peptides and that centipede venoms are likely to be an excellent source of novel insecticidal compounds. Indeed, according to the ArachnoServer database (35), the LD$_{50}$ values obtained for the most potent insecticidal centipede venom peptides purified in the current study (5 pmol/g) are similar to the LD$_{50}$ values reported for the most potent insecticidal spider venom peptides discovered to date.

$\mu$-SLPTX-Ssm1a was found to be a potent inhibitor of vertebrate TTX-S Na$_V$ channels, and we therefore presume that inhibition of insect Na$_V$ channels is the basis of its insecticidal activity. Historically, Na$_V$ channels have been the most commonly exploited insecticide target, being the site of action of insecticides such as pyrethroids, indoxacarb, and DDT. In contrast, none of the currently available chemical insecticides target K$_V$ channels. Thus, the demonstration that three of the insecticidal peptides we isolated target K$_V$ channels in DRG neurons is particularly interesting because it could lead to the identification of novel insecticide targets if block of insect K$_V$ channels is the basis of their insecticidal activity.

**Conclusions and Future Directions**—The current work has provided the first molecular evidence for the presence of disulfide-rich neurotoxins with structural and functional diversity in centipede venoms. Although these peptide neurotoxins are functionally similar to the disulfide-rich neurotoxins found in the venoms of SS animals in that they modulate the activity of voltage-gated ion channels, in almost all cases the primary structures of the centipede venom peptides are unique. This represents an interesting case of convergent evolution in which different venomous animals have evolved different molecular strategies for targeting the same ion channels in prey and predators.

The current demonstration of novel disulfide-rich peptides in centipede venoms indicates that they may provide a new source of novel bioactive molecules. There is increasing interest in venoms as a source of novel pharmaceuticals (36), and centipede venoms may therefore have a role to play in venom-based drug discovery programs. Considering their diversity (>3000 species), their roles as a major component of terrestrial ecosystems throughout temperate and tropical regions, and their position as active predators, feeding on small invertebrates that are sometimes larger than themselves (37), the authors hope that the current study will stimulate more interest in the venoms of this hitherto neglected group.

In this study, we provided evidence for the presence of unique disulfide-rich neurotoxins in the venom of a centipede from the order Scolopendromorpha. Centipedes comprise five orders, four within the class Pleurostigmophora (including Scolopendromorpha) and the sole order Scutigeromorpha within the class Notostigmophora (17). To understand the evolution of these neurotoxins, it will be important in future studies to determine whether they are widespread in other members of the class Pleurostigmophora and, in particular, whether they are also present in scutigeromorphs. Unfortunately, however, it is difficult to obtain sufficient centipede venom for detailed structure-function characterization of individual toxins. However, recent technological developments such as the miniaturization of bioassays and improvements in the sensitivity of mass spectrometry and NMR spectroscopy (38) should facilitate study of the molecular diversity of centipede venoms. Moreover, as demonstrated here, transcriptomic studies can provide detailed insight into the venom proteome of centipedes using only a limited number of specimens.

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