Articles

δ-Conotoxin GmVIA, a Novel Peptide from the Venom of *Conus gloriamaris*†

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ABSTRACT: A novel peptide toxin, δ-conotoxin GmVIA, was purified from the venom of *Conus gloriamaris*, a mollusc-hunting snail. It consists of 29 amino acids, including six Cys residues:

VKPCRKEGQLCDPIFQNCCRGWNCVLFCV-OH

The pattern of disulfide connectivity (4–19, 12–24, and 18–29) is the same as for the ω-conotoxins, which are Ca^{2+} channel ligands. However, the peptide does not compete with ω-conotoxin for binding to membrane preparations from frog, rat, and chick brain. Instead, initial electrophysiological results suggest that the peptide induces action potential broadening in molluscan neurons by slowing down Na^+ current inactivation. Synthetic δ-conotoxin GmVIA was prepared by solid-phase methods and appeared identical in all respects to the natural material. The chromatographic behavior of native and reduced δ-conotoxins is quite remarkable, suggesting that the disulfides form a core which forces hydrophobic residues to point out toward the solvent.

The venoms of predatory molluscs belonging to the genus *Conus* have proven to be a rich cornucopia of small peptides (conotoxins), which are increasingly useful reagents in neuroscience. Conotoxins are used as subtype-specific ligands for receptors and ion channels (Myers et al., 1993; Olivera et al., 1990).

The shells of *Conus* snails ("cone shells") have long been popular collectors' items. Of all such shells, perhaps the most famous is that of the Glory-of-the-Sea, *Conus gloriamaris*. For several hundred years, this shell was an extraordinary rarity, and when available it was sold with old-master paintings and jewelry in the major auctions of Europe. At one time, it was perhaps one of the most highly valued of all natural history specimens. The great beauty and delicacy of the pattern of *C. gloriamaris* (shown in Figure 1), its rarity, and the fact that after the mid-19th century no specimens had been collected for perhaps a hundred years all added to the cachet of this particular *Conus* species.

Only in the last few decades has it been discovered that *C. gloriamaris* lives in rather deep water across the biotically rich marine geographic province called the "Indo-Pacific arc" (from the Philippines to New Guinea). One locality for the collection of *C. gloriamaris* has been the abyssal waters of Manila Bay; our laboratories were able to obtain a few specimens of *C. gloriamaris* from trawlers at this locality. The venom ducts of *C. gloriamaris* proved to be exceptionally large and to carry significantly more venom than do those of most *Conus* species. *C. gloriamaris* appears related (primarily by its shell patterns) to the mollusc-hunting cones, particularly the *Conus textile* group.

FIGURE 1: Shell pattern of *Conus gloriamaris*, the Glory-of-the-Sea cone: a close-up of the shell of the highly venomous marine snail.

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In this report, we describe the purification of δ-conotoxin GmVIA, the first conotoxin characterized from *C. gloriamaris*. Quantitatively, this peptide is present in unusually large amounts. The toxin was purified, its amino acid sequence and disulfide bridge pattern were established, and a preliminary electrophysiological characterization was carried out. The purified peptide was examined in a molluscan system, and preliminary evidence suggests that the toxin induces action potential broadening by slowing the rate of sodium current inactivation. Synthetic peptide, prepared by solid-phase methods, showed the same biological activity.

MATERIALS AND METHODS

Materials. Specimens of *C. gloriamaris* were dissected, and the venom ducts were removed. The venom was squeezed and scraped from the ducts as described by Cruz et al. (1976) and then lyophilized. Tris(2-carboxyethyl)phosphine (TCEP)[1] was synthesized by the method of Burns et al. (1991).

Crude Venom Extraction. Lyophilized venom (200 mg) was first suspended in 5 mL of 0.1% TFA and 40% acetonitrile and soaked for 10 min over ice with occasional stirring and sonication. The solution was then centrifuged for 5 min using a bench-top microfuge and the supernatant was saved. The extraction procedure was repeated two more times with the same solvent and twice with 5 mL of 0.1% TFA and 90% acetonitrile. Supernatants from all extractions were combined and stored over ice while awaiting further purification. The pellet was dried and weighed.

Peptide Purification by HPLC. Pooled venom extracts were subjected to reversed-phase HPLC using a C_8 Aquapore semipreparative column (7.0 × 250 mm; 4 mL/min). Secondary purification was carried out on a C_{18} Vydac column (218TP54, 4.6 × 250 mm; 1 mL/min). HPLC buffers were (A) 0.1% TFA in water and (B) 0.09% TFA in 90% acetonitrile. For both semipreparative and analytical runs, peptides were eluted with a linear gradient of 1% buffer B increase/min. The C_{18} Vydac analytical column was also used for purifying partially reduced intermediates and alkylated peptides during disulfide bridge analysis.

Amino Acid Sequence. Disulfides were reduced by incubating equal volumes of peptide solution and 8 mM TCEP in 0.25 M Tris, pH 8, for 20 min at room temperature. A single product was obtained by reversed-phase HPLC on the C_{18} Vydac analytical column. The reduced peptide was alkylated by the addition of 1 μL of 4-VP/100 μL of peptide solution. After incubation for 20–30 min in the dark, the solution was diluted to reduce acetonitrile concentration, and the pyridylethylated peptide was repurified by reversed-phase HPLC. The eluted peptide was adsorbed onto Biobrene-treated glass fiber filters, and the amino acid sequence was analyzed by automated Edman degradation on an ABI Model 477A instrument. Sequence analyses were carried out by Dr. Robert Schackmann of the Utah Regional Cancer Center.

Disulfide bridge analysis was carried out by the partial reduction method of Gray (1993). δ-Conotoxin GmVIA in HPLC column effluent was added to an equal volume of 20 mM TCEP in 0.17 M sodium citrate, pH 3, and incubated for 5 min at 64 °C. The partially reduced species were purified by HPLC and were immediately frozen in solution (pH 2) at −80 °C to prevent disulfide exchange. For convenience, and to minimize loss of peptide, all intermediates were kept in the HPLC effluent without drying them down completely. Partially reduced peptides were alkylated by squirting 200 μL of thawed peptide solution into a supersaturated solution of IAM (100 mg in 200 μL of 0.5 M Tris–acetate, pH 8, containing 2 mM EDTA), while the latter was mixed rapidly. After 20–30 s, the reaction was quenched by adding 450 μL of 0.5 M citric acid solution. Alkylated peptides were purified by HPLC and were then subjected to complete reduction and further alkylation by 4-VP as described previously.

Solid-Phase Peptide Synthesis. δ-Conotoxin GmVIA was synthesized by the two-stage strategy employed for ω-conotoxin MVIID (Monje et al., 1993). The protected peptide resin was built using standard fmoc chemistry, couplings being carried out with equimolar amounts of amino acid derivative, DCC, HOBT. All amino acids were purchased from Bachem (Torrance, CA), and side chains were protected as follows: Arg (pmc), Asn (trt), Asp (*t*-bu), Gln (trt), Glu (*t*-bu), and Lys (boc). Cys residues 4, 18, 19, and 28 were protected by trt, while Cys residues 11 and 24 were protected by acm.

At the completion of synthesis, the terminal fmoc group was removed by standard treatment with piperidine/NMP (20% by volume). Peptide was removed from the resin by treatment for 2 h at 20 °C with TFA/H_2O/ethanedithiol/phenol/thioanisole (90/5/2.5/7.5/5 by volume), and the whole mixture was filtered rapidly into *t*-butyl methyl ether at −10 °C. Linear peptide, retaining protection only on Cys11 and Cys24, was collected as a pellet after centrifugation and was washed once with *t*-butyl methyl ether. The pellet dissolved readily in 60% acetonitrile containing 0.1% TFA; an ether layer that separated was discarded. Peptide solution was diluted with 0.1% aqueous TFA before application to a 2.5- × 25-cm column of Vydac C_{18}. Elution was carried out at 20 mL/min, using a gradient of acetonitrile (27–50%) in 0.1% TFA. The major peptide-containing fraction was diluted with 42% acetonitrile, the pH was adjusted to 7.0 with NaOH, and the solution was stirred overnight at room temperature. This procedure oxidized Cys residues 4, 18, 19, and 29, generating three bicyclic isomers which were isolated by HPLC. In small-scale trials, one of these gave nativelike material after oxidation with 2 mM I_2 in 10% TFA (10 min, 20 °C, followed by a quench with 30 mM ascorbic acid). Oxidation was then carried out preparatively on that isomer, and the tricyclic peptide was isolated by HPLC.

Bioassays and Biological Activities. Local garden snails, *Helix aspersa*, weighing 3–5 g were chosen for bioassays, because they were readily available. In the presence of food, the snails became aroused and no longer stayed in the shells. Once they were fully active and out of the shells, the snails were put on ice to slow down their activity. Under these conditions the heads tend to remain extended, allowing easy injection of 10–40-μL aliquots of toxin fractions. The injection, similar to intracranial injection in the common mouse bioassay (Olivera et al., 1984), was near the cerebral ganglion where nerves are heavily localized. For comparison, intracranial injection into two-week-old mice was done with doses of 20–60 nmol.

Electrophysiology. Isolated neurons from *Aplysia californica* or *Aplysia oculifera* were cultured as previously described (Schacher & Proshansky, 1983; Hasson et al., 1993).

[1] Abbreviations: acm, acetamidomethyl; ASW, artificial sea water; boc, *tert*-butyloxycarbonyl; CAM, carboxyamidomethyl; CNS, central nervous system; DCC, dicyclohexylcarbodiimide; fmoc, fluorenylmethyloxycarbonyl; HEPES, *N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulfonic acid); HOBT, hydroxybenzotriazole; HPLC, high-performance liquid chromatography; IAM, iodoacetamide; LSIMS, liquid secondary ionization mass spectrometry; NMP, *N*-methylpyrrolidone; NMR, nuclear magnetic resonance; PCBS, potassium conductance blocking solution; PE, pyridylethyl; pmc, pentamethylchromansulfonyl; *t*-bu, *tert*-butyl; TCEP, tris(2-carboxyethyl)phosphine; TFA, trifluoroacetic acid; trt, trityl; 4-VP, 4-vinylpyridine.

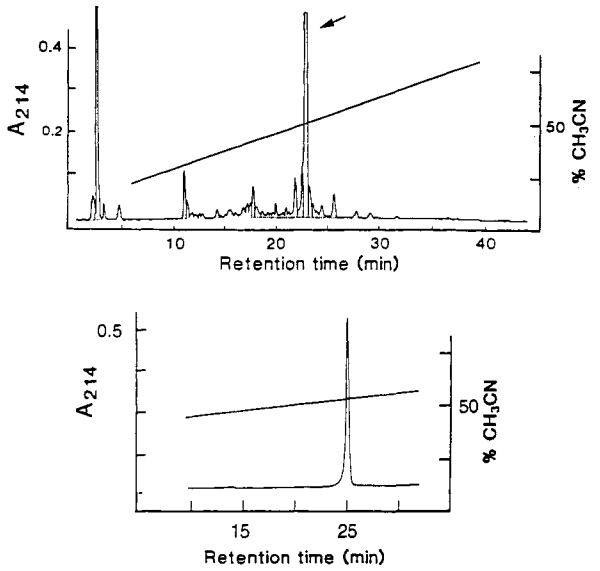

FIGURE 2: (Top) Reversed-phase HPLC chromatogram of the *Conus gloriamaris* venom extracted with 0.1% TFA and 90% acetonitrile. Ten microliters of the extracted solution (total volume 25 mL) from 200 mg of the lyophilized venom was eluted from the analytical C_8 Aquapore column. The GmVIA peptide, approximately 35% of the absorbance area under all peaks, is indicated with an arrow. (Bottom) Reversed-phase HPLC chromatogram of GmVIA after repurification on the analytical C_{18} Vydac column.

The neurons were cultured at very low densities to prevent synaptic interactions among them. Passive and active membrane properties of the cultured neurons were studied using conventional intracellular recording and stimulation techniques. Briefly, the cell body of a cultured neuron was impaled by a microelectrode filled with 2 M KCl (4–10 MΩ resistance). The electrode was used for both current injection and voltage recordings. Analysis of the resting potential, input resistance, and action potential amplitude and shape was carried out in artificial sea water (ASW) composed of 460 mM NaCl, 10 mM KCl, 11 mM $CaCl_2$, 55 mM $MgCl_2$, and 10 mM HEPES, pH 7.6. The toxin for electrophysiological experiments was dissolved in ASW containing 10 mg/mL bovine serum albumin and was applied to the bathing solution to reach a final concentration of 0.3–0.75 μM.

Competitive Binding with ω-Conotoxin GVIA. The procedures for membrane preparation and binding assay were essentially as previously described by Cruz and Olivera (1986) except that NaCl was used instead of choline chloride in the wash medium of the binding assay. Different concentrations of the peptide and ω-conotoxin GVIA were preincubated with the membrane preparation for 30 min on ice before the addition of ^{125}I-labeled GVIA.

RESULTS

Purification and in Vivo Activity of δ-Conotoxin GmVIA. Crude venom was prepared from *C. gloriamaris* ducts as described under Materials and Methods and fractionated by HPLC on a C_8 Aquapore column (Figure 2, top). A large number of absorbance peaks were observed, one of which accounted for approximately 35% of the total absorbance of the soluble fraction (arrow in figure). This major component was further purified using a C_{18} Vydac analytical column. As shown in Figure 2 (bottom), this material was eluted as an apparently homogeneous component from that column. The peptide is markedly hydrophobic compared to most *Conus* peptides, eluting at 49% acetonitrile/0.1% TFA.

Because *C. gloriamaris* is believed to be a snail-hunting cone, our initial *in vivo* bioassay used local garden snails.

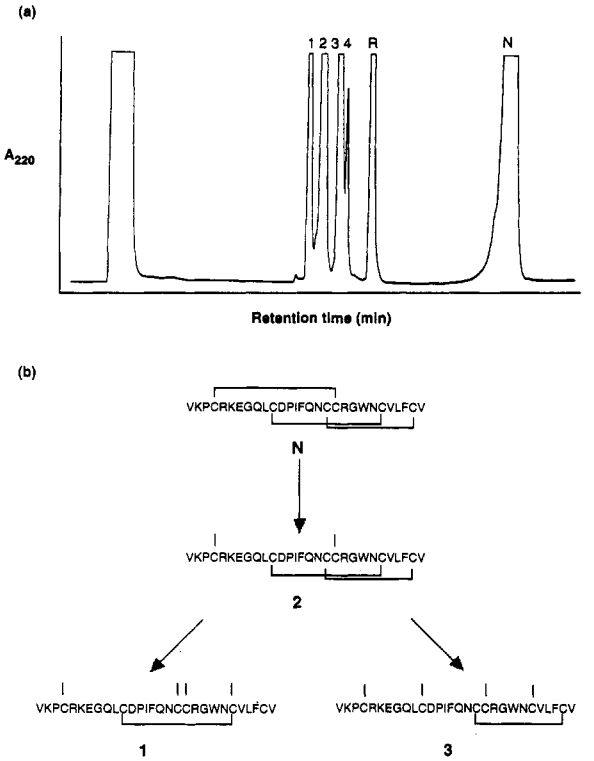

FIGURE 3: (a) Reversed-phase HPLC chromatogram of δ-conotoxin GmVIA, partially reduced by TCEP (10 mM, pH 3, 5 min, 64 °C). The absorbance peaks labeled 1–4 correspond to the partially reduced peptides PR1–PR4; N and R correspond to the native and completely reduced peptides, respectively. An analytical C_{18} Vydac column was used for purification; flow rate was 1 mL/min and an acetonitrile gradient was increased 1%/min. (b) Schematic diagram of disulfide bridge arrangements in native and partially reduced peptides.

Volumes of peptide solution between 10 and 40 μL were injected in the head region, near the cerebral ganglion. Injection of approximately 20 nmol of purified δ-conotoxin GmVIA induced retraction of the head and body into the shell; this was followed by secretion of viscous green slime and a convulsive undulation into and out of the shell. Biological effects on garden snails were detectable at a dose of 1.25 nmol/g and very obvious at 2 nmol/g. No apparent biological activity was observed when a much greater dose of peptide (10 nmol/g) was injected peritoneally into mice.

Amino Acid Sequence and Disulfide Bridges of δ-Conotoxin GmVIA. Analysis of pyridylethylated peptide by standard Edman chemistry gave the sequence Val-Lys-Pro-Cys-Arg-Lys-Glu-Gly-Gln-Leu-Cys-Asp-Pro-Ile-Phe-Gln-Asn-Cys-Cys-Arg-Gly-Trp-Asn-Cys-Val-Leu-Phe-Cys-Val. Completeness of the sequence was indicated by mass analysis using LSIMS. The observed $(M + H)^+$ was consistent with the above sequence, having a free carboxyl at the C-terminus and three disulfide bridges [monoisotopic; observed $(M + H)^+$ = 3352.5; theory, 3352.51]. Thus the peptide has 29 amino acids, and its six cysteine residues are arrayed in the pattern (–C–C–CC–C–C–) typical of the ω-conotoxins and δ-conotoxin TxVIA. Both δ-conotoxins lack the C-terminal amidation characteristic of most conotoxins.

The unusually high abundance of peptide in venom enabled us to analyze its disulfide bridge connectivity, using partial reduction by TCEP at pH 3 (Gray, 1993). No reduction was observed at room temperature, but a useful spectrum of products was obtained after 5 min at 64 °C (Figure 3a). Completely reduced (R) and native (N) peptides are indicated in this chromatogram, as well as four partially reduced

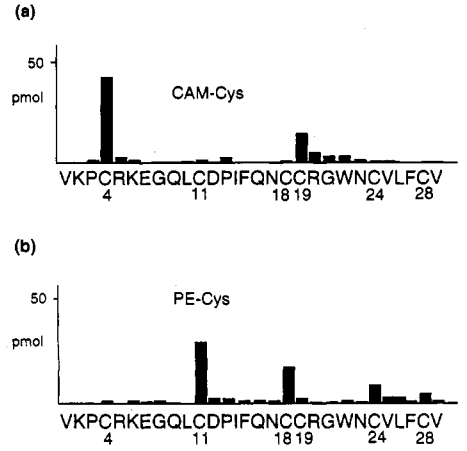

FIGURE 4: Sequence analysis of partially reduced and alkylated GmVIA peptide PR2 (Figure 3a). The y-axis represents yield (picomoles) of the appropriate Cys derivative at each stage of analysis, and the x-axis represents the amino acid sequence of the peptide. CAM-Cys and PE-Cys were quantitated separately to distinguish cysteines that are alkylated with different chemical agents.

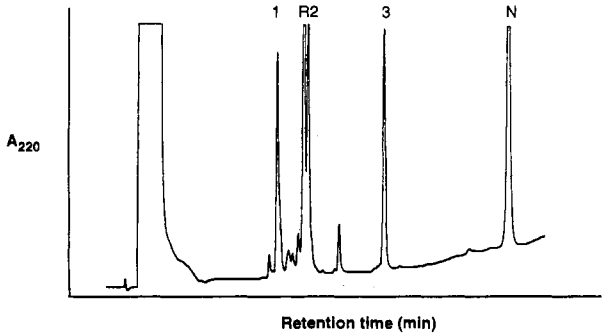

FIGURE 5: Reversed-phase HPLC chromatogram of δ-conotoxin TxVIA, partially reduced by TCEP (10 mM, pH 3, 5 min, 64 °C). Absorbance peaks are labeled as per Figure 3, with three partially reduced peaks.

intermediates (PR1–PR4). Peptides PR1, -2, and -3 were further purified and analyzed. Reaction with iodoacetamide, using the rapid alkylation protocol as described under Materials and Methods (Gray, 1993), proceeded with minimal rearrangement of the disulfides. All remaining Cys residues were pyridylethylated following full reduction and alkylation with 4-VP. Thus, every cysteine residue was converted either to Cys(CAM), if it had been part of a bridge which was reduced, or to Cys(PE), if it had been part of a bridge which had initially remained intact. The intermediates were then sequenced to locate the two labels.

Analysis of PR2 is shown in Figure 4, which reveals labeling of Cys4 and Cys19 with CAM and of the remaining cysteines with PE. This indicates that a bridge linking Cys4 and Cys19 was the only one which had been reduced. PR1 and PR3 proved to be monocyclic peptides in which only Cys11–Cys24 and Cys18–Cys29 remained intact, respectively. These results form a completely consistent set, indicating that the bridges are linked sequentially [4–19, 11–24, and 18–29] in the same pattern as that observed with the ω-conotoxins. The structure of δ-conotoxin GmVIA is shown in Figure 3b, along with the major pathway for reduction by TCEP.

For comparison, the disulfide bridge connectivities of the related peptide δ-conotoxin TxVIA (Hillyard et al., 1989; Fainzilber et al., 1994) were also analyzed by the same methods. A generally similar pattern was obtained after partial reduction (Figure 5), and analysis of intermediates 2 and 3 was sufficient to establish that the disulfide connectivity

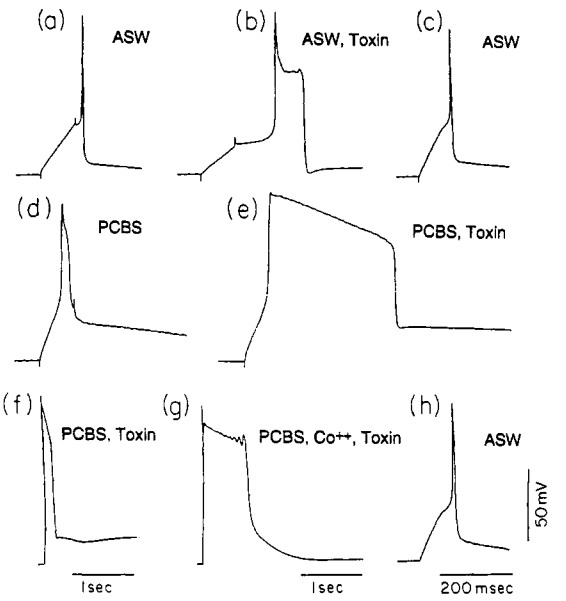

FIGURE 6: Electrophysiology: action potential prolongation by δ-conotoxin GmVIA. In ASW GmVIA peptide prolongs the action potential duration. (a) Control; (b) 2 min after bath application of 0.5 μM GmVIA peptide; (c) recovery of the action potential duration following wash by ASW. Following blockage of the potassium conductances by PCBS (PCBS is an ASW in which potassium ions were substituted by Cs^+; it contains 50 mM tetraethylammonium chloride and 0.1 mM 3,4-diaminopyridine), bath application of 0.5 μM toxin prolongs the action potential duration (e and f). In the presence of PCBS and the toxin, blockage of Ca^{2+} conductance by 9 mM Co^{2+} induces further spike broadening to over 1 s (g). After washing of the neuron by ASW, the spike duration recovers (h). The voltage and time scales for all experiments are as shown in (h) except for (f) and (g), where the time scale is as indicated.

was the same as that of δ-conotoxin GmVIA.

Chemical Synthesis of δ-Conotoxin GmVIA. Construction of the protected peptide resin proceeded smoothly. A single major product was obtained after deprotection and cleavage, accounting for 53% of the total absorbance at 220 nm. Air oxidation of this material gave the expected three bicyclic peptides, with a pattern similar to that obtained for ω-conotoxin MVIID (Monje et al., 1993). Treatment of the second of these bicyclic peptides resulted in approximately 60% conversion to a product having the same elution time as natural peptide. Coinjection experiments verified that the two products were indistinguishable by this method. Bioassays in the snail showed that synthetic peptide was equipotent with the natural.

Electrophysiology. A preliminary study of the electrophysiological effects of purified toxin was carried out on isolated *Aplysia* neurons. Changes in the resting potential, input resistance, and action potential amplitude and shape upon addition of the toxin were assessed. The purified toxin revealed significant effects at final concentrations of 0.3–0.75 μM. Within 10–60 s after bath application of the toxin, quiescent neurons fired spontaneously (not shown). Concomitantly the action potential duration increased by 1–2 orders of magnitude (Figure 6b), extending in many experiments to over 250 ms. The changes in membrane excitability and action potential duration induced by the toxin were completely reversible upon washing of the neuron with ASW (Figure 6b,h).

Toxin-induced prolongation of the action potential was still observed when K^+ and Ca^{2+} conductances were blocked, suggesting that GmVIA's effect is most likely due to a decrease in the rate of sodium current inactivation. For instance, in

		Charges		
δ-Conotoxins		+	-	Net
GmVIA	VKPCRKEGQLCDPIFQNCCRGWNC---VLFCV^	5	3	+2
TxVIA	WCKQSGEMCNLLDQNCCDGY-C--IVLVCT^	2	4	-2
ω-Conotoxins				
GVIA	CKSOGSSCSOTSYNCCRS--CNOYTKRCY*	5	0	+5
MVIIA	CKGKGAKCSRLMYDCCTGS-C-RSGK-C*	7	1	+6
MVIIC	CKGKGAPCRKTMYDCCSGS-CGRRGK-C*	8	1	+7
MVIID	CQGRGASCRKTMYNCCSGS-C-NRG-RC*	6	0	+6
SVIA	CRSSGSOCGVTSI-CCGR--C-YRGK-CT*	5	0	+5

FIGURE 7: Comparison of δ-conotoxin GmVIA with δ-conotoxin TxVIA (*Conus textile*) and selected ω-conotoxins: ω-conotoxin GVIA is from *Conus geographus*, MVIIA, B, C, and D are from *Conus magus*, and SVIA is from *Conus striatus*. The sequences are shown to align cysteine residues to highlight the cysteine frames. References: GmVIA, this work; TxVIA, Hillyard et al. (1989); GVIA, Olivera et al. (1984); MVIIA, Olivera et al. (1987); MVIIC, Hillyard et al. (1992); MVIID, Monje et al. (1993); SVIA, Ramilo et al. 1992). ^ indicates free carboxyl; * indicates amide.

the experiment of Figure 6d, K^+ conductances were blocked by using ASW in which KCl was replaced by CsCl. In addition, the solution contained 50 mM tetraethylammonium chloride and 0.1 mM 3,4-diaminopyridine (osmolarity of the solution was restored by reducing the NaCl concentration to 410 mM). We refer to this solution as potassium conductance blocking solution or PCBS. Under these conditions, bath application of 0.5 μM toxin prolonged the spike duration (Figure 6e,f). In the presence of the toxin and PCBS, bath application of Co^{2+} to block Ca^{2+} current (final concentration of 8 mM) increased spike duration even further (Figure 6g). This is most likely due to blockage of residual Ca^{2+}-dependent K^+ conductances, which contribute to the repolarization of the action potential.

These observations, together with preliminary whole-cell patch-clamp studies performed recently by us (Hasson et al., manuscript in preparation), indicate that the mechanism underlying the toxin effect is slowing down of sodium current inactivation, rather than changes in Ca^{2+} or K^+ currents.

Competitive Binding with ω-Conotoxin GVIA. At concentrations up to 5 μM of the test peptide, δ-conotoxin GmVIA did not compete with ^{125}I-labeled ω-conotoxin GVIA on brain membrane preparations from frogs, chicks, and rats. Positive controls with unlabeled ω-conotoxin GVIA gave the expected level of competition: 25 nM unlabeled toxin displaced approximately 90% of ^{125}I-GVIA, and 250 nM competed out approximately 98% of label.

DISCUSSION

In this report, we have described the major peptide toxin from venom of the mollusc-hunting cone *C. gloriamaris*. Structurally, δ-conotoxin GmVIA belongs to the general class containing the ω-conotoxins from fish-hunting snails and the "King-Kong" peptide from another mollusc-hunting snail, *C. textile* (Hillyard et al., 1989), now called δ-conotoxin TxVIA (Fainzilber et al., 1994). In Figure 7, the sequences of δ-conotoxin GmVIA and δ-conotoxin TxVIA are compared with those of selected ω-conotoxins. Several features emerge at this level: (1) Cys residues are arranged similarly in all peptides, with minor variation in loop sizes. We have shown that the similar arrangement of cysteines in the linear sequences is reflected in an identical disulfide bonding pattern in the δ- and ω-conotoxins. (2) There is great diversity in the amino acids at almost all positions other than Cys residues. (3) The ω-conotoxins are hydrophilic and carry a high net positive charge, while the δ-conotoxins are very hydrophobic and have little net charge.

The various conotoxins are synthesized as larger polypeptide precursors. The mature ω-conotoxins and δ-conotoxins are peptides of 25–30 amino acids; an analysis of cDNA clones has revealed precursors 70–80 amino acids in length [see Woodward et al. (1990) and Colledge et ál. (1992)]. Although there is little amino acid sequence homology (except for the Cys residues) between mature δ-conotoxin and ω-conotoxin sequences, there is significant homology in the precursor signal sequence region. It has been hypothesized that the precursors are the folding modules which guide specific disulfide linkages to be formed in the mature toxin (Woodward et al. 1990; Olivera et al., 1990; Colledge et al., 1992). The precursor structure for δ-conotoxin GmVIA has not yet been determined, but we predict that it will be very similar to those of δ-conotoxin TxVIA and the ω-conotoxins. We are currently investigating this problem, and at this point it appears likely that the two groups of toxins are part of a single superfamily.

As with the ω-conotoxins, amino acid sequence divergence is extensive between δ-conotoxins TxVIA and GmVIA, but they share features that make them a distinct subgroup. In contrast to most of the neuroactive conotoxins, including the ω-conotoxins, the two δ-conotoxins are not strongly basic. They are also remarkably hydrophobic, while the majority of conotoxins are very polar.

Despite the close relationship of the δ-conotoxins to the ω-conotoxins, it is clear that they have different physiological targets. The ω-conotoxins inhibit voltage-gated Ca^{2+} channels, distinguishing various subtypes. In contrast, the δ-conotoxins are without effect on Ca^{2+} channels: our results show that they do not compete for binding with ω-conotoxin GVIA and do not induce the shaking syndrome in mice characteristic of the ω-conotoxins.

The preliminary electrophysiological results presented here demonstrate that δ-conotoxin GmVIA prolongs spike duration by slowing the inactivation kinetics of the sodium current, and thus at the gross physiological level it appears to have effects similar to those of δ-conotoxin TxVIA (Hasson et al., 1993). Detailed electrophysiological studies to be published elsewhere (A. Hasson et al., manuscript in preparation) will provide further evidence that δ-conotoxin GmVIA specifically targets Na^+ channels and prolongs the action potential duration by slowing down the sodium current inactivation. The data clearly indicate that there are significant differences between GmVIA and TxVIA at a detailed mechanistic level. In many ways, this is not so surprising because of the tremendous sequence divergence between GmVIA and TxVIA.

Biologically active δ-conotoxin GmVIA has been chemically synthesized, demonstrating that the biological activity is not due to contaminants. A different family of *Conus* peptides, the μ-conotoxins, is known which also affects Na^+ channels. However, these have a different disulfide framework, are channel blockers specific for the muscle subtype, and like the ω-conotoxins are highly basic molecules. Given the very

different chemical character of δ-conotoxins, it seems likely that their site of action on the Na^+ channel is quite distinct.

Just as the ω-conotoxins are a major feature of many fish-hunting cone venoms, the δ-conotoxins may be a major feature of molluscivorous venoms, particularly of those species that are related to *C. textile* and *C. gloriamaris*. In both cases, the δ-conotoxins are the major toxins found in these venoms; in *C. gloriamaris*, δ-conotoxin GmVIA is quantitatively more dominant than any other toxin found so far in a *Conus* venom, comprising fully 35% of all the peptide absorbance present.

This hydrophobic character of δ-conotoxins is very pronounced and exhibited in an unusual manner: with both GmVIA and TxVIA the native folded peptides are more hydrophobic than the linear molecules without disulfide bonds, as indicated by their much later elution on reversed-phase HPLC. Such results indicate that the disulfide bonding framework may be forcing hydrophobic residues outward toward the medium. These exposed hydrophobic residues would presumably be important for binding the target Na^+ channels, perhaps from the side exposed to the lipid bilayer. Clearly, an NMR structure of the δ-conotoxins is necessary to verify this unusual structural hypothesis.

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