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The Spasmodic Peptide Defines a New Conotoxin Superfamily†

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ABSTRACT: We purified and characterized a peptide from the venom of *Conus textile* that makes normal mice assume the phenotype of a well-known mutant, the spasmodic mouse. This "spasmodic" peptide has 27 amino acids, including two γ-carboxyglutamate (Gla) residues. A cDNA clone encoding the precursor for the peptide was identified; a γ-carboxylation recognition signal sequence (γ-CRS) is present in the $-1 \rightarrow -20$ region of the peptide precursor. Both the γ-CRS and the position of the Gla residues in the mature toxin are notably different from other Gla-containing conopeptides. The spasmodic peptide has a novel disulfide framework and distinct signal sequence which together define a new P-superfamily of conopeptides. A cDNA encoding another member of the P-superfamily was identified from a different species, *Conus gloriamaris*.

The small, conformationally constrained peptides found in the venoms of marine snails belonging to the genus *Conus* are widely used tools in neuroscience (*1*−*3*); several are even being developed for therapeutic use (*4, 5*). In the literature, experiments using *Conus* peptides are described in over 1800 published papers. Several peptides that have become important pharmacological tools for neuroscientists were originally isolated on the basis of the unusual behavioral symptomatologies that they elicited after injection into the CNS of mice (*6*). Thus, the ω-conotoxins, which specifically block presynaptic calcium channels and are widely used to inhibit synaptic transmission, were identified initially as "shaker" peptides since they caused a distinctive shaking syndrome in the injected mouse (*7*). Another group of peptides purified on the basis of the distinct behavioral symptomatology elicited were the "sleeper" peptides, now called conantokins, which caused a sleep-like state in young mice and a hyperactivity syndrome characterized by constant climbing in mature mice (*5, 8, 9*). These proved to be the first peptide ligands specific for the NMDA receptor and are being explored for possible clinical use as anticonvulsants (*5*). Thus, *Conus* peptides that elicit unusual behavioral syndromes in mice are of particular interest since the neurobiochemical mechanisms that underlie the behavior may be quite novel.

In this paper, we describe biological and molecular data for an unusual peptide from *Conus textile* venom that elicits a distinctive behavioral symptomatology in mice. Upon injection of this venom component, to be called the "spasmodic peptide", normal mice are converted into behavioral phenocopies of a well-known mutant, the spasmodic mouse (*10, 11*).

In addition, we show that the spasmodic peptide and a related peptide from another *Conus* species share a distinctive Cys pattern, one not previously seen in any conotoxin family.

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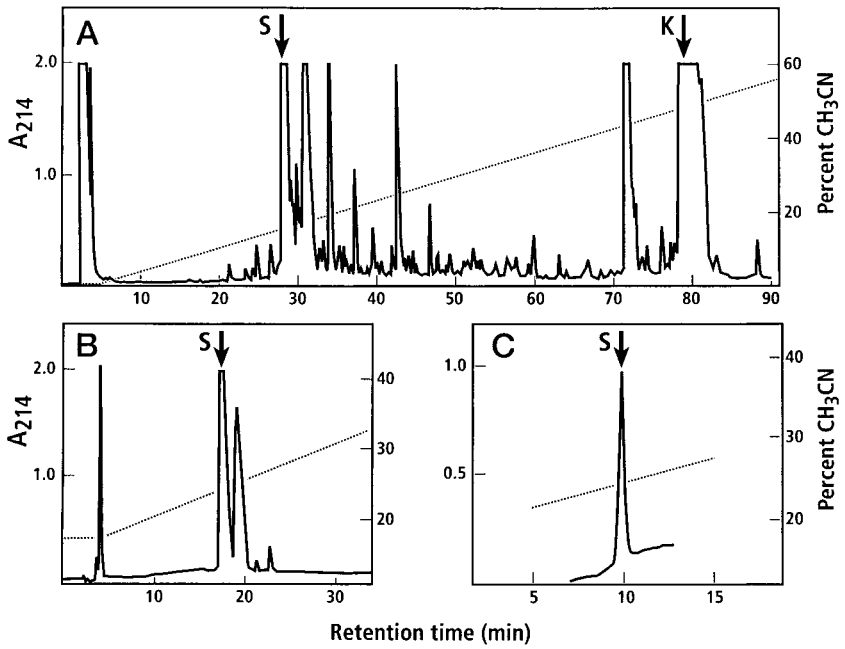

FIGURE 1: Purification of the spasmodic peptide from C. *textile* venom. An extract of crude venom was applied on a Bio-Gel column as previously described (*12*). Panel A shows the HPLC chromatogram of a size fraction that also contained the King Kong peptide or δ-conotoxin TxVIA (K arrow). The peak marked by the S arrow in panel A was chromatographed on a Vydac reverse-phase C_{18} column. The gradient of acetonitrile in 0.1% TFA is indicated by the dashed line. The major peak in panel B was rerun on the same column−buffer system to obtain the pure peptide shown in panel C, which was reduced and alkylated and used for amino acid sequence analysis.

Thus, not only does the spasmodic peptide elicit a noteworthy behavioral effect on mice, but it also is the prototypic peptide for a novel superfamily of conotoxins.

METHODS

Purification of the Spasmodic Peptide. The spasmodic peptide was purified from *C. textile* venom by two different methods:

Purification I. Freeze-dried *C. textile* venom was extracted with 0.2 M ammonium acetate and then fractionated in a Bio-Gel column as described by Hillyard et al. (*12*). The spasmodic peptide and the previously described King Kong peptide (δ-conotoxin TxVIA) came from the same size fraction. As shown in Figure 1, the succeeding steps involved HPLC runs on a reverse-phase C_{18} column eluted with a gradient of acetonitrile (ACN)[1] in 0.1% trifluoroacetic acid (TFA). The relative positions of the spasmodic peptide and the King-Kong peptide are indicated in panel A.

Purification II. Lyophilized *C. textile* venom from specimens collected in the Philippines (125 mg) was extracted sequentially with 10 mL each of H_2O, 20% ACN, 40% ACN, 60% ACN, and 90% ACN. The mixture was sonicated for three 30-s periods over ice water and centrifuged at 5000*g* for 5 min; the supernatants were stored at −20 °C. The crude venom extract was applied to a preparative scale reversed-phase HPLC; the extract (20 mL) was diluted to 350 mL with 0.1% TFA solution and applied to a C_{18} Vydac preparative column (22.0 × 250 mm). Fractions were eluted at 20 mL/min with a linear gradient of 0.1% TFA in water and 0.09% TFA in 60% acetonitrile. Further purification of the peptide used C_{18} Microsorb MV and C_{18} Vydac analytical columns at a gradient of 0.23% acetonitrile/min and a flow rate of 1 mL/min. The effluents were monitored at 220 nm, fractions were collected in propylene tubes, and aliquots were assayed for biological activity.

Reduction and Alkylation. The purified peptide (1.2 nmol) was reduced with dithiothreitol (DTT) and alkylated with 4-vinylpyridine. The pH of the peptide solution was adjusted to 8 with 0.5 M tris(hydroxymethyl)amino methane, and DTT was added to a final concentration of 10 mM. The solution was flushed with nitrogen, incubated at 65 °C for 15 min, and cooled to room temperature. Five microliters of 4-vinylpyridine was added per milliliter of solution; the mixture was left in the dark at room temperature for 25 min and then diluted with 500 μL of 0.1% TFA. The mixture was applied on an analytical C_{18} Microsorb MV HPLC column, which was eluted using 0.1% TFA and 0.085% TFA in 90% acetonitrile (B90) as limiting buffers. The alkylated peptide was recovered by first eluting the column for 50 min with 12% buffer B90 to remove most of the reaction byproducts before applying a gradient of 12−90% buffer B90 over 78 min at a flow rate of 1 mL/min. A blank reaction (without peptide) was run on HPLC for comparison.

Sequencing. The alkylated peptide (300 pmol) was sequenced by standard Edman methods using Applied Biosystem model 492 sequenator (DNA/Peptide Facility, University of Utah). The 3-phenyl-2-thiohydantoin derivatives were identified by HPLC. The sequence was confirmed by mass spectrometry.

Cloning the Spasmodic Peptide. On the basis of the amino acid sequence of the isolated spasmodic peptide from *C. textile*, we designed oligonucleotide primers for PCR amplification of the corresponding cDNA from a directionally cloned cDNA library (*13*). Three oligonucleotide primers

[1] Abbreviations: 5HT, 5-hydroxytryptamine (serotonin); AA, amino acid; ACN, acetonitrile; DTT, dithiothreitol; i.c., intracranial; TFA, trifluoroacetic acid.

with degenerate nucleotide sequences were synthesized. Primer 1, 5′ CCR TTI ACI GCI CCR CAI CC 3′; primer 2, TGR CAI SWR TTR TTR CAI CC 3′; and primer 3, ATR CAR TGI SWY TCR CAR TC 3′ (where I = inosine, R = A and G, Y = C and T, S = G and C, and W = A and T) represent sequences complementary to the coding sequences at the C-terminus, central, and N-terminus of the peptide, respectively. Primary amplification was carried out using a vector-specific 5′ oligonucleotide and primer 1 in a 1605 Air Thermo-Cycler (Idaho Technology, Idaho Falls, ID). The product was reamplified using the 5′ vector-specific primer and primer 2 and then electrophoresed on an agarose gel. The major product isolated using Qiaquick gel extraction kit (Qiagen, Valencia, CA) was ligated to pGEM-T vector DNA (Promega, Madison, WI) and used to transform *Escherichia coli* DH5α. The nucleic acid sequence of DNA inserted into pGEM-T was determined at the DNA Sequencing Facility at the University of Utah. An oligonucleotide primer corresponding to 5′ sequences was thus obtained, and a vector-specific 3′ primer was used to PCR amplify the entire clone. The amplified DNA was cloned and sequenced. The entire sequence of spasmodic cDNA was assembled from the overlapping sequences and is predicted to contain the amino acid sequence of the mature spasmodic toxin.

cDNA corresponding to the spasmodic peptide from *C. gloriamaris* was obtained by PCR amplification of DNA isolated from a directionally cloned *C. gloriamaris* cDNA library. Oligonucleotide primers corresponding to the 5′ and the 3′ untranslated regions of the previously isolated *C. textile* spasmodic peptide cDNA were used. The amplified DNA was cloned and its sequence determined as described above.

γ-Glutamyl Carboxylase Assay. The peptide corresponding to the −20 to −1 region of the spasmodic peptide precursor, linked at its C-terminus to the pentapeptide FLEEL-NH_2, was synthesized by Dr. R. Schackmann, DNA Peptide Facility, Huntsman Cancer Center, University of Utah (supported by Grant NCICA42014). The identity of the peptide was confirmed using ESI-MS. Partially purified γ-carboxyglutamate carboxylase was prepared as described by Stanley et al. (*14*). The assay was carried out as described by Bandyopadhyay et al. (*15*), except that the spasmodic peptide pro region (−20 to −1)·FLEEL-NH_2 was used as the substrate for the reaction. Experiments were done in triplicate, and the data were fit to a single-site binding model and analyzed using GraphPad Prism from GraphPad Software, Inc. (San Diego, CA).

Bioassay. The biological activity of the peptide was determined using 9−15-day-old mice. Approximately 5−290 pmol (per gram body weight) of the lyophilized samples dissolved in normal saline solution were injected i.c. (intracerebral) into mice. Control mice were injected with equal volume of normal saline solution containing dissolved residue (if any) of the corresponding lyophilized column buffer. After injection, the mice were returned to their cages and observed for the onset of any abnormal behavior.

Siamese fighting fish were injected in the dorsal muscle with 10 μL of the saline solution of the peptide and observed for suppression of aggressive behavior when placed in mirrored aquaria. Likewise, control fish were injected with normal saline solution using 26-gauge insulin syringes. Each fish was observed for 1 h or longer depending on the activity.

RESULTS

Purification of the Spasmodic Peptide. The spasmodic peptide was initially detected as an early-eluting major peak from crude *Conus textile* venom, which was notable for a characteristic suite of symptoms observed after i.c. injection into mice. Within a certain dose range, injected mice were hypersensitive to sensory input and, when either touched or exposed to auditory stimulation, became hyperexcitable to the point where seizure-like symptoms could be induced. Since this symptomatology is characteristic of mutant mice strains carrying either the spasmodic (*10, 11*) or spastic (*16*) mutation, we trivially refer to this peptide as the "spasmodic peptide", terminology used for the remainder of this paper. The purification of the spasmodic peptide from crude *Conus textile* venom is shown in Figure 1. The peptide was identified through the various purification steps by following the spasmodic symptomatology described above.

When the purified peptide was injected into mice, even a dose of ∼10 pmol/g was sufficient to induce running in circles and hyperactivity. At higher doses (50 pmol/g), the mice exhibited running and climbing symptoms for close to 1 h. Between 130 and 150 pmol/g, characteristic "spasmodic" symptomatology was elicited. A hand clap would make mice jump high and start running rapidly. When exposed to a loud hand clap, or if the cage cover were dropped, the mice lost motor control and exhibited seizure-like symptoms from which they eventually recovered. At the highest doses tested (>250 pmol/g body weight), after the characteristic spasmodic symptomatology, lethality occurred. Injection of a similar dose range intramuscularly into fish elicited no unusual symptomatology.

Biochemical Characterization of the Spasmodic Peptide; cDNA cloning. The amino acid sequence of two batches of purified peptide was determined using standard Edman chemistry. Purified peptide was reduced and alkylated (see Methods), and a single unequivocal sequence could be assigned through 27 Edman steps, except that no assignment could be made for positions 8 and 13: GCNNSCQXHSDCXSHCICTFRGCGAVN (where X meant no assignment could be made). However, a trace of Glu was detected at the two unassigned positions, characteristic of residues that have been posttranslationally modified from glutamate to γ-carboxyglutamate. The presence of γ-carboxyglutamate in the peptide was directly confirmed by alkaline hydrolysis as previously described (*8*).

To definitively establish the sequence of the spasmodic peptide, a cDNA clone encoding the spasmodic peptide was identified and characterized from a *Conus textile* library (*13*), and a mass spectrometric analysis was carried out. The data in Table 1 show the predicted sequence for the open reading frame from the cDNA clone. This sequence corresponds with amino acid sequence analysis, except for positions 8 and 13 where the cDNA sequence predicts a glutamate residue at both positions, consistent with positions 8 and 13 being γ-carboxyglutamate (Gla) in the mature gene product. The cDNA sequence also predicts that the C-terminal asparagine is amidated (since the C-terminal glycine of the spasmodic peptide precursor would be processed to give an amidated C-terminus in the mature peptide). All of the data taken together are consistent with the following sequence assignment for the spasmodic peptide:

Table 1[a]

A. Nucleotide and predicted amino acid sequence derived from the cDNA (clone Tx9.1) encoding the spasmodic peptide precursor from *C.textile*.

```
1/1                                             31/11
ATG CAT CTG TCA CTG GCA CGC TCA GCT GTT TTG ATG TTG CTT CTG CTG TTT GCC TTG GGC
M   H   L   S   L   A   R   S   A   V   L   M   L   L   L   L   F   A   L   G
61/21                                           91/31
AAC TTT GTT GTG GTC CAG TCA GGA CAG ATA ACA AGA GAT GTG GAC AAT GGA CAG CTC ACA
N   F   V   V   V   Q   S   G   Q   I   T   R   D   V   D   N   G   Q   L   T
121/41                                          141/51
GAC AAC CGC CGT AAC CTG CAA TCG AAG TGG AAG CCA GTG AGT CTC TAC ATG TCA CGA CGG
D   N   R   R   N   L   Q   S   K   W   K   P   V   S   L   Y   M   S   R   R
181/61                                          211/71
GGT TGT AAC AAT TCT TGC CAG GAG CAT TCC GAT TGC GAA TCC CAT TGT ATT TGC ACG TTT
G   C   N   N   S   C   Q   E   H   S   D   C   E   S   H   C   I   C   T   F
241/81
AGA GGA TGC GGA GCT GTT AAT GGT TGA
R   G   C   G   A   V   N   G   *
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B. Comparison of predicted amino acid sequence of prepro- and mature spasmodic peptides from *C. textile* and *C. gloriamaris* deduced from cDNA clones Tx9.1 and Gm9.1. Amino acid differences are underlined.

```
                    -60                 -40                 -20
C. textile          MHLSLARSAVLMLLLLFALGNFVVVQSGQITRDVDNGQLTDNRRNLQSKWK
C. gloriamaris      MHLSLARSAVLMLLLLFALGNFVVVQSGLITRDVDNGQLTDNRRNLQTEWN
                              1                   20
C. textile          PVSLYMSRRGCNNSCQEHSDCESHCICTFRGCGAVNG*
C. gloriamaris      PLSLFMSRRSCNNSCQSHSDCASHCICTFRGCGAVNG*

Mature peptides:
C. textile (tx9a)       GCNNSCQγHSDCγSHCICTFRGCGAVN#
C. gloriamaris          SCNNSCQSHSDCASHCICTFRGCGAVN#
```

[a] In concordance with the nomenclature designated by McIntosh et al. (*3*), we designate all peptides with six nonadjacent Cys residues (C−C−C−C−C−C) as Cys framework 9—all peptides with this framework shall have 9 or IX (the latter when the mechanism has been determined). Thus, the spasmodic peptide (from *C. textile* venom) is technically referred to as peptide tx9a, encoded by Tx9.1. When the mechanism is known, it might be renamed by a designation such as "π-conotoxin IXA". The cDNA clone from *C. gloriamaris* is Gm9.1.

```
     5         10        15        20        25
GCNNSCQγHSDCγSHCICTFRGCGAVN-NH2
```

(where γ is γ-carboxyglutamate)

Also consistent with the sequence assignment above are the mass spectrometry analyses. Using LDMS, a value of 2955.1 was obtained; an electrospray determination gave a mass of 2955.0. The predicted mass of the mature peptide shown above is 2955.03.

Evidence for a γ-Carboxylation Recognition Signal (γ-CRS) Sequence in the −1 to −20 Region of the Precursor for the Spasmodic Peptide. The presence of γ-carboxyglutamate in the spasmodic peptide suggests that a γ-CRS is docking the γ-carboxylase enzyme at a site N-terminal to the glutamate residues to be posttranslationally modified. It was previously established that the −1 to −20 region of the conantokin-G precursor (another γ-carboxylated conopeptide) contains functional recognition signal sequences (*15*). To test whether the spasmodic peptide precursor from *C. textile* similarly contains a γ-carboxylation recognition signal sequence in its −1 to −20 region, a peptide chimera was synthesized. The −1 to −20 region from the spasmodic peptide precursor was attached to a model γ-carboxylation substrate FLEEL. FLEEL, initially designed as a substrate for mammalian γ-glutamyl carboxylase (*17*), has previously been used for the study of *Conus* carboxylase (*14*, *15*, *18*, *19*). The γ-carboxylation of FLEEL could then be assessed in the absence and presence of the −1 to −20 region of the spasmodic peptide. The results are shown in Figure 2. Clearly, the presence of the −1 to −20 spasmodic peptide region does indeed increase the affinity for the targeted FLEEL sequence by over 30-fold. The estimated apparent K_m values in the absence and presence of propeptide are 1.4 $\times 10^{-4}$ and 4.7 $\times 10^{-6}$ M, respectively. These results provide evidence for a γ-CRS in the propeptide region of the spasmodic peptide precursor.

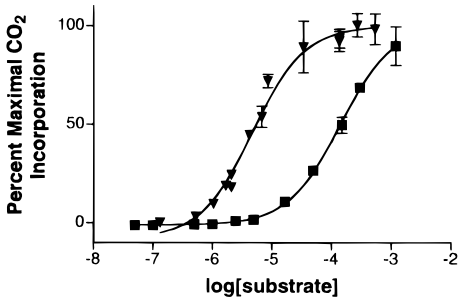

FIGURE 2: Enzymatic carboxylation of spasmodic peptide pro-(−20 to −1)·FLEEL-NH_2 (▼) and FLEEL (■). The $^{14}CO_2$ incorporated into the substrates is expressed as percent of maximal incorporation at the highest substrate concentration used in the experiment. The estimated apparent K_m values in the presence and absence of propeptide are 4.7 and 140 μM, respectively. It should be noted that saturating amounts of FLEEL were not achieved so the apparent K_m of 140 μM is probably a low estimate. The data were fit to a single site binding model.

A Conotoxin Related to the Spasmodic Peptide from Conus gloriamaris. In an attempt to characterize other potential members of the spasmodic peptide family, an analysis of other *Conus* species for cDNA clones related to the spasmodic peptide precursor was carried out. The predicted amino acid sequence of an open reading frame in a cDNA clone from another molluscivorous *Conus* species, *C. gloriamaris*, is also shown in Table 2.

The putative sequence of the *Conus gloriamaris* peptide exhibits a striking level of sequence identity to the spasmodic peptide from *C. textile*. However, in *Conus gloriamaris* peptide the two γ-carboxyglutamates of the spasmodic peptide of *C. textile* are mutated to serine and alanine. Functional differences between the two peptides have not yet been defined, since the peptide from neither *C. textile* nor *C. gloriamaris* has been successfully chemically synthesized. However, the results so far indicate that the spasmodic peptide family may be a particularly favorable group to investigate structure/function for peptides containing γ-carboxyglutamate residues.

DISCUSSION

In this paper, we describe the characterization of the spasmodic peptide, a novel γ-carboxyglutamate-containing peptide from *C. textile* venom. The biological activity of the spasmodic peptide is notable in that, when the peptide is

Table 2

A. Comparison of γ-CRS-containing (-1 to -20) sequences of three Gla-containing peptides.

	γ-CRS (-20 to -1)	Mature Peptide
Spasmodic (*C. textile*)	DNRRNLQSKWKPVSLYMSRR	GCNNSCQγHSDCγSHCICTFRGCGAVN#
Conantokin-G (*C. geographus*)	GKDRLTQMKRILKQRGNKAR	GEγγLQγNQγLIRγKSN#
tx5a (*C. textile*)	PLSSLRDNLKRTIRTRLNIR	γCCγDGW*CCT^{s}AAO

B. Comparison of P-, O- and T-superfamily peptides.

	Signal Sequence (-60 to -20)	Mature Conotoxin
P-Superfamily (spasmodic peptide)		
C. textile	MHLSLARSAVLMLLLLFALGNFVVVSG	GCNNSCQγHSDCγSHCICTFRGCGAVN#
C. gloriamaris	MHLSLARSAVLMLLLLFALGNFVVVSG	SCNNSCQSHSDCASHCICTFRGCGAVN#
O-Superfamily (δ-conotoxins)		
C. textile	MKLTCMMIVAVLFLTAWTFATA	WCKQSGEMCNLLDQNCCDGYCIVLVCT
C. gloriamaris	MKLTCMMIVAVLFLTAWTFATA	VKPCRKEGQLCDPIFQNCCRGWNCVLFCV
T-Superfamily (tx5a)		
C. textile	MRCFPVFIILLLLIASAPCFDA	γCCγDGW*CCT^{s}AAO

γ, γ-carboxyglutamate; W*, bromotryptophan; T^{s}, O-glycosylated threonine; O, hydroxyproline; #, C-terminal amidation.

Summary of Cys frameworks:

P-Superfamily	C—C—C—C—C—C
O-Superfamily	C—C—CC—C—C
T-Superfamily	CC——CC

injected into the CNS of mice, it converts normal mice into phenocopies of two well-known mutant mouse strains, the spastic and spasmodic mice (*10*, *11*, *16*, *20*−*22*). The distinctive symptomatology that these mouse mutants exhibit is an extreme hypersensititivy to sensory stimuli, causing a loss of motor control and seizure-like symptoms. Injection of the spasmodic peptide into normal mice elicits this sensory hypersensitivity symptomatology. For this reason, we have adopted the common and more descriptive name "spasmodic peptide"; technically, using the nomenclature previously proposed (*3*) the peptide is designated tx9a, and the cDNA encoding it is designated Tx9.1.

At this time, the high affinity molecular target of the spasmodic peptide remains unknown. It has been shown that the spastic and spasmodic mouse mutant phenotypes result from a deficit in glycine receptors; preliminary tests to determine whether the spasmodic peptide competed with radiolabeled strychnine for binding to cloned glycine receptors were negative (M. Becker and H. Betz, unpublished results). However, the possibility that the spasmodic peptide targets a very specific glycine receptor subtype and/or acts on the glycine receptor noncompetitively has not been eliminated.

The spasmodic peptide is one of an increasing number of *Conus* peptides shown to contain the unusual amino acid γ-carboxyglutamate (Gla). Gla is formed from Glu in a vitamin K-dependent γ-carboxylation reaction; the enzyme recognizes potential substrates through a γ-carboxylation recognition signal (γ-CRS). The presence of a γ-CRS in the −1 to −20 propeptide region of the spasmodic peptide precursor was directly demonstrated. However, there is little apparent sequence homology to other *Conus* peptide γ-carboxylation recognition sequences (see Table 2A) (*15*, *23*). γ-Carboxylation recognition sequences were originally defined for the mammalian enzyme (*24*−*26*). A comprehensive list of mammalian γ-CRSs has been compiled by Furie et al. (*27*), and the role of the amino acids in the interaction has been compiled by Stanley et al. (*28*).

Conotoxins, the multiply disulfide-bonded peptides from *Conus* venoms, can be grouped into families and superfamilies—members of a family share a distinctive disulfide bonding pattern *and* target a homologous set of sites in a family of receptor or ion channel proteins. Among the best-characterized families of *Conus* peptides are the ω-conotoxins, α-conotoxins, and δ-conotoxins (which target voltage-gated calcium channels, nicotinic acetylcholine receptors, and voltage-gated sodium channels, respectively). The ω- and δ-conotoxins share the same disulfide pattern and belong to the O-superfamily, while the α-conotoxins have a different disulfide pattern and belong to the A-superfamily.

A characteristic of previously defined conotoxin families is the remarkable hypermutation that occurs as *Conus* species diverge (*29*). By molecular cloning, we were able to identify another member of the spasmodic peptide family from another species, *Conus gloriamaris*, which shared a high degree of sequence identity with the *C. textile* spasmodic peptide. A sequence comparison for the two species analyzed of both the spasmodic peptide and the δ-conotoxin families is shown in Table 2. It is immediately obvious that although there is striking interspecific sequence divergence for the δ-conotoxins (with only 26% sequence identity in the non-Cys AA between δ-conotoxins from the two species) (*12*, *30*), there is little sequence variation seen in the spasmodic peptides (>85% sequence identity in non-Cys AA). Thus, in contrast to the unusual level of interspecific hypermutation observed with most conotoxin superfamilies (*13*, *29*), the spasmodic peptide family may be more conserved, at least within the mollusc-hunting clade of *Conus* species.

In addition to its distinctive biological activity, the spasmodic peptide is noteworthy in that so far no other conotoxin has been described with the same arrangement of Cys residues. The only other *Conus* peptide reported in the literature that has multiple disulfide bonds with no adjacent pairs of cysteine residues is σ-conotoxin GVIIIA, a peptide that targets the $5HT_3$ receptor (*31*) and belongs to the S-superfamily. Although there are some similarities in the spacing of the cysteine residues, the spasmodic peptide cannot be considered to be part of the S-superfamily of conotoxins. First, σ-conotoxins have 10 Cys residues while the spasmodic peptide has 6; furthermore, the spasmodic peptide does not share any sequence homology in its precursor sequence with the σ-conotoxins. Thus, the spasmodic peptide is the first member of a novel superfamily of conotoxins, which we designate the P-superfamily. As is true of other conotoxin superfamilies, the P-superfamily can be recognized by both a unique signal sequence and distinct arrangement of Cys residues. These defining features are summarized in Table 2 SectionB and compared to other conotoxin superfamily members from the two *Conus* species from which P-superfamily members have been identified.

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