Isolation and Pharmacological Characterization of Cannitoxin, a Presynaptic Neurotoxin from the Venom of the Papuan Taipan (Oxyuranus scutellatus canni)

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ABSTRACT

The Papuan taipan (Oxyuranus scutellatus canni) is widely distributed throughout much of Papua New Guinea. Although neurotoxicity is a major symptom of envenomation, no neurotoxins have been isolated from this venom. Using a series of size exclusion chromatography steps, we report the isolation of cannitoxin, a presynaptic neurotoxin (44,848 Da) that represents approximately 16% of the whole venom. The toxin displayed high phospholipase A₂ (PLA₂) activity (330 ± 5 μmol/min/mg) and caused concentration-dependent (11–66 nM) inhibition of indirect (0.2 ms; 0.1 Hz; supramaximal V) twitches of the chick biventer cervicis nerve-muscle preparation without effecting nicotinic receptor agonists. Prior addition of CSL Taipan antivenom (5 U/ml) or inhibition of phospholipase A₂ activity by incubation with 4-bromophenacyl bromide prevented the inhibition of twitches. Cannitoxin is composed of three different subunits, α, β, and γ, with the possibility of two β isomers. However, only the α subunit displayed in vitro neurotoxic activity of its own. Thus, cannitoxin is similar in structure and pharmacology to taipoxin, which has been isolated from the closely related Australian species O. scutellatus scutellatus (coastal taipan).

Three species of taipans (genus Oxyuranus) have been identified and are considered to be the world’s most venomous snakes (Sutherland and Tibballs, 2001). These are the Australian coastal taipan (Oxyuranus scutellatus), inland taipan (Oxyuranus microlepidotus), and Papuan taipan (Oxyuranus scutellatus canni) that are found throughout the northern coastal region of Australia (Worrell, 1970), central Australia (Sutherland and Tibballs, 2001), and in the low-lying and mountainous terrain of Papua New Guinea (O’Shea, 1996), respectively. The rank order of potency of the venoms based on murine LD₅₀ (s.c.) data is as follows: O. microlepidotus (0.025 mg/kg) (Broad et al., 1979) > O. scutellatus canni (0.0505 mg/kg) (Sutherland and Tibballs, 2001) > Oxyuranus scutellatus scutellatus (0.099 mg/kg) (Broad et al., 1979). However, t₉₀ (i.e., time to produce 90% inhibition of indirect twitches) data obtained in the chick biventer cervicis nerve-muscle (CBCNM) preparation indicated that O. scutellatus scutellatus venom is more neurotoxic than that of O. scutellatus canni venom (Crachi et al., 1999b). They also showed that CSL Taipan antivenom (CTPV) was markedly more effective in neutralizing the neurotoxic effects of the Papuan or coastal taipan venoms than that of the inland taipan (Crachi et al., 1999b).

Presynaptic neurotoxins act at the motor nerve terminal to either facilitate (e.g., dendrotoxin) or inhibit (e.g., β-bungarotoxin, taipoxin, and paradoxin) the release of neurotransmitter. All of the presynaptic inhibitors have phospholipase A₂ (PLA₂) activity (Harris, 1991). Taipoxin and paradoxin were isolated from the venoms of O. scutellatus scutellatus (Fohlman et al., 1976) and O. microlepidotus, respectively. Taipoxin is a ternary complex of three subunits, α-, β-, and γ-taipoxin, that exists in a stoichiometry of 1:1:1 held together by noncovalent interactions. The α subunit is the only subunit with toxic activity on its own. The β subunit is neutral and exists in two isoforms (i.e., β₁ and β₂), which are interchangeable in the complex. The γ subunit is the largest of the three subunits, and it seems to be glycosylated (Fohl-
man et al., 1976). Paradoxin has a similar structure to taipoxin (Fohlman, 1979); however, this neurotoxin has been less well studied, and no reports of the presence of two β isoforms have been located in the literature.

Envenoming by the Papuan taipan is a significant health problem in Papua New Guinea, with 70 to 80 patients being admitted to the Port Moresby General Hospital alone each year (Connolly et al., 1995). Symptoms of envenoming include local tender lymphadenopathy, abdominal pain, coagulopathy, and neurotoxicity (Connolly et al., 1995). However, research on the venom of the Papuan taipan has been limited to a basic pharmacological examination (Crachi et al., 1999a,b), and no neurotoxins have been isolated so far. In particular, it is not known whether the venom contains a presynaptic neurotoxin similar to paradoxin and taipoxin, which have been isolated from the two closely related species. This study reports the isolation and characterization of cannitoxin, a presynaptic neurotoxin from the venom of the Papuan taipan.

Materials and Methods

All chromatography separations were performed using a Shimadzu (Kyoto, Japan) high-performance liquid chromatography system (LC-10ATvp pump and SPD-10AVP detector).

Size Exclusion Chromatography

Freeze-dried venom was dissolved in ammonium acetate buffer (0.1 M; pH 5.0), and insoluble material was removed by centrifugation at 5000 g for 5 min. The supernatant was applied to a Superdex G-75 column (13 mm × 10 × 300 mm; GE Healthcare, Little Chalfont, Buckinghamshire, UK) equilibrated with ammonium acetate buffer. The sample was eluted at a flow rate of 0.5 ml/min. The purified component was rerun under the same conditions to ensure purity. The eluant was monitored at 280 nm.

Separation of Cannitoxin Subunits Using Reverse Phase-High Performance Liquid Chromatography

The freeze-dried, purified component was reconstituted in Milli-Q water (Millipore Corporation, Billerica, MA) and applied to a Phenomenex (Torrance, CA) Jupiter analytical C18 column (150 × 2 mm; 5 μm; 300 A) after equilibrating with solvent A (0.1% trifluoroacetic acid). The sample was eluted with the following gradient conditions of solvent B at a flow rate of 0.2 ml/min: 0 to 20% over 5 min, 20 to 60% in 40 min, and then 60 to 80% over 5 min (Wickramaratna et al., 2004). The eluant was monitored at 280 and 214 nm.

Molecular Mass Determination

Molecular mass was determined by both size exclusion chromatography and mass spectrometry.

Size Exclusion Chromatography on Nondenaturing Media

The molecular mass of the complex was determined by gel filtration on a Superdex G-75 column equilibrated with ammonium acetate buffer (0.1 M; pH 5.0). The column was calibrated with the series of known standards (6500–66,000 Da) using the molecular weight marker kit for gel filtration chromatography (lot no. 093K9307; MWGF-70; Sigma-Aldrich, St. Louis, MO). The eluant was monitored at 280 nm, and a flow rate of 0.6 ml/min was used. Void volume (V0) of the column was determined by running blue dextran, and the elution volume (Ve) was calculated for each molecular weight marker before injecting the purified component (0.5 mg). The molecular weight of the toxin was determined from a plot of log (mol. wt.) versus Ve/V0 ratio.

Mass Spectrometry. MALDI-TOF MS analysis was performed with an Applied Biosystems (Foster City, CA) Voyager-DE STR BioSpectrometry Workstation. The instrument was operated in positive polarity in linear mode using sinapinic acid matrix (Agilent Technologies, Palo Alto, CA) for low-resolution protein analysis. Matrix (1 μl) was spotted on the sample plate and allowed to air-dry; sample (1 μl) diluted in acetonitrile/water (1:1) containing 0.1% (v/v) formic acid was subsequently spotted on dried matrix and allowed to air-dry. Data from 500 laser shots (337-nm nitrogen laser) were collected, and the signal was averaged and processed with the instrument manufacturer’s Data Explorer software.

CBCNM Preparation

Chickens (4–10-day-old males) were sacrificed with CO2, and both biventer cervicis cervico-muscle preparations were dissected. These were mounted under 1-g resting tension in 5-ml organ baths containing physiological salt solution of the following composition: 118.4 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO4, 1.2 mM KH2PO4, 2.5 mM CaCl2, 25 mM NaHCO3, and 11.1 mM glucose. The solution was maintained at 34°C and bubbled with carbogen (95% O2 and 5% CO2).

Motor nerves were stimulated every 10 s (0.2-ms duration) at supramaximal voltage using a Grass S88 stimulator (Harvey et al., 1994). D-Tubocurarine (10 μM) was added, and the subsequent abolition of twitches confirmed the selective stimulation of nerves. Responses to nerve stimulation were re-established by thorough washing. Contractile responses to acetylcholine (ACh; 1 mM for 30 s), carbachol (CCh; 20 μM for 60 s), and KCl (40 mM for 30 s) were obtained in the absence of stimulation (Harvey et al., 1994). The preparations were then equilibrated for at least 30 min with continuous nerve stimulation (as described above) before the addition of toxin. In all experiments, toxin (11–66 nM), subunits (100 nM), or venom (10 μg/ml) was left in contact with the tissue for 5 h if total twitch blockade did not occur. At the conclusion of the experiment, responses to ACh, CCh, and KCl were obtained as described previously. Time taken to reduce the amplitude of the indirect twitches by 90% (t90) was calculated to provide a quantitative measure of neurotoxicity (Crachi et al., 1999a).

Where indicated, CTPV (5 U/ml) was added 10 min before the addition of toxin. Reversibility of the venom was tested by adding TPAV (5 U/ml) at t90 after the addition of venom (10 μg/ml). Anti-venom was left in contact with the tissue for 2 h.

Determination of PLA2 Activity

PLA2 activity of the venom, cannitoxin, and cannitoxin subunits was determined using a colorimetric assay kit (Cayman Chemical, Ann Arbor, MI) designed to test the activity of secretory PLA2s. This assay uses the 1,2-dithio analog of diheptanoyl phosphatidylcholine, which serves as a substrate for PLA2 enzymes. Free thiols generated following the hydrolysis of the thioester bond at the sn-2 position by PLA2 are detected using 5,5′-dithio-bis-(2-nitrobenzoic acid). Color changes were monitored using a CERES900C microplate reader (Bio-Tek Instruments, Winooski, VT) at 405 nm, sampling every minute for a 5-min period. PLA2 activity was expressed as micromoles of phosphatidylcholine hydrolyzed per minute per milligram of enzyme.

PLA2 Inhibition with 4-Bromophenacyl Bromide

PLA2 activity of venom was inhibited by alkylation with 4-bromophenacyl bromide (4-BPB). Toxin (11 nM) made up in sodium cacodylate-HCl buffer (0.1 M; pH 6.0) and 4-BPB made up in acetone were added to produce a final concentration of 1.8 mM (Abe et al., 1977; Bell et al., 1998; Crachi et al., 1999a). Vials containing the above-mentioned mixture were incubated for 16 h at 30°C. Where indicated, toxin made up in sodium cacodylate-HCl buffer incubated with acetone was used as a vehicle control for 4-BPB (Wickramaratna et al., 2003).
N-Terminal Amino Acid Sequence Determination

Purified peptides were loaded into the sequencing chamber of a Prociise N-terminal amino acid sequencer (Applied Biosystems), and the amino acid sequence was determined (Edman degradation, phenylthiohydantoin derivatization chemistry, and separation of derivatized amino acids by RP-HPLC) using the manufacturer’s recommended methods and reagents.

Chemicals and Drugs

The following drugs were used: acetylcholine chloride, bovine serum albumin, carbamylcholine chloride (carbachol), d-tubocurarine, 4-bromophenacyl bromide, ammonium acetate, molecular weight marker kit (6500–66,000), and cacodylic acid (Sigma-Aldrich); KCl (Ajax Chemicals, Sydney, Australia); trifluoroacetic acid (Auszep, Melbourne, Australia); acetonitrile (Ajax Finechem, Seven Hills, New South Wales, Australia); CSL Taipan antivenom (CSL Ltd., Melbourne, Australia); and acetone (BDH Chemicals, Victoria, Australia).

Analysis of Results and Statistics

In isolated tissue experiments, responses were measured via a Grass PTO3 force displacement transducer and recorded on a PowerLab system (ADInstruments Pty Ltd., Castle Hill, Australia). Twitch height and contractile responses to agonists were expressed as a percentage of the corresponding value before the addition of toxin. Statistical difference was determined by a one-way analysis of variance (ANOVA) on the twitch height at the 300-min time point and on the contractile responses to exogenous agonists. All ANOVAs were followed by a Bonferroni post hoc test, and statistical significance was indicated where \( P < 0.05 \).

Results

Chick Biventer Studies: Whole Venom

Venom from the Papuan taipan displays postsynaptic neurotoxic activity (Crachi et al., 1999a) that is prevented by CSL Taipan antivenom (Crachi et al., 1999b). In the current study, taipan antivenom (5 U/ml) added at \( t_{90} \) (44 ± 8.2 min) failed to reverse or prevent further inhibition of indirect twitches over a period of 2 h (Fig. 1a). However, antivenom restored the response of the tissue to exogenous ACh and CCh (Fig. 1b). This confirmed the presence of a presynaptic neurotoxin and indicated that only the postsynaptic neurotoxic effects of the venom can be reversed by taipan antivenom. Therefore, we decided to purify and characterize the presynaptic neurotoxin.

Size Exclusion Chromatography

Cannitoxin was isolated from the venom of the Papuan taipan following successive separations by size exclusion chromatography. Initial fractionation on a Superdex G-75 column produced six main peaks (Fig. 2a). Screening in the chick biventer cervicis nerve-muscle preparation indicated the presence of four subunits, two of which are likely to represent isomers as evidenced by similar molecular mass resemblance as well as the split molecular mass of cannitoxin was determined from a plot of log mol. wt. versus the sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) profile of the venom. The subunits of cannitoxin were further fractionated using an Effect of taipan antivenom (5 U/ml) after the addition of venom (10 \( \mu g/ml \)) or vehicle at \( t_{90} \) (arrow) on indirect twitches (a) and contractile responses (b) of the CBCNM preparation. *, \( P < 0.05 \), significantly different compared with vehicle control; one-way ANOVA; \( n = 4 \).

RP-HPLC

The subunits of cannitoxin were further fractionated using an RP-HPLC Phenomenex Jupiter analytical column. This produced four main peaks (Fig. 2c) that were analyzed based on the N-terminal amino acid sequences as well as molecular masses, and they were assigned as the following subunits (see below): \( \alpha \) (13,824 Da), \( \beta_1 \) (13,242 Da), \( \beta_2 \) (13,276 Da), and \( \gamma \) (17,762 Da). The molecular masses were determined by MALDI-TOF (see below) analysis and confirmed by electrospray ionization-MS.

Molecular Mass Determination

Size Exclusion Chromatography in Nondenaturing Media. Molecular mass was determined by size exclusion chromatography on a Superdex G-75 column and calibrated with the following protein standards: BSA (66,000 Da), carbonic anhydrase (29,000 Da), cytochrome \( c \) (12,400 Da), and aprotinin (6500 Da). The \( V_e \) of the column was determined as 7.56 ml using blue dextran (2,000,000 Da), and the \( V_o \) of cannitoxin was calculated as 10.15 ml. The molecular mass of cannitoxin was determined from a plot of log mol. wt. versus corresponding \( V_e/V_o \) ratio (of the protein standards described above) to be 45,000 Da (Fig. 3).

Mass Spectrometry. Initial MALDI-TOF analysis of the complex protein indicated the presence of four subunits, two of which are likely to represent isomers as evidenced by similar molecular mass resemblance as well as the split...
peaks in MALDI spectra (data not shown). MALDI analysis also indicated a possible glycosylation in one of the subunits.

In addition, individual subunits purified by RP-HPLC were analyzed by MALDI-TOF. The subunits were found to be of the following molecular masses: 13,242 Da (\(\beta_2\)), 13,276 Da (\(\beta_1\)), 13,824 Da (\(\alpha\)), and 17,762 Da (\(\gamma\)). MALDI-TOF analysis showed the \(\gamma\) subunit (17,762 Da) to be heterogeneously glycosylated (data not shown), confirming the data mentioned above. These masses are in agreement with those observed upon initial MALDI-TOF analysis of the protein complex and were also confirmed by electrospray ionization-MS. The sum of the molecular masses of \(\alpha\), \(\gamma\), and \(\beta_1\) or \(\beta_2\) subunits results in an average mass of 44,848 Da, which is in agreement with that determined by size exclusion chromatography.

**N-Terminal Amino Acid Sequence**

Partial N-terminal amino acid sequence of the isolated subunits of cannitoxin was determined using Edman degradation (Table 1). These were compared with the protein sequences of the corresponding subunits of taipoxin at the National Center for Biotechnology Information database using the BLAST service. The subunits showed sequence identity in the order \(\beta_1\) (100%) > \(\gamma\) (86%) > \(\alpha\) (85%), based on the partial N-terminal sequence. Since the other subunit is structurally similar to \(\beta_1\), we identified this subunit as \(\beta_2\). The N-terminal sequence of \(\beta_2\) taipoxin was not listed in the database or in the literature; hence, comparisons cannot be made.

**Chick Biventer Studies**

**Cannitoxin.** Cannitoxin (11–66 nM) caused concentration-dependent inhibition of indirect twitches in the CBCNM preparation (Fig. 4a), but it had no effect on the response of the tissue to ACh, CCh, or KCl (Fig. 4b), indicating its action at the presynaptic terminal. Cannitoxin incubated with 4-BPB, or TPAV added before the addition of cannitoxin, resulted in prevention of the toxin-induced inhibition of indirect twitches (Fig. 4a), but it had no significant effect on the response of the tissue to exogenous agonists (Fig. 4b). Vehicle (i.e., BSA) and cannitoxin in the presence of vehicle (i.e., acetone) had no effect on the agonist responses (data not shown).

**Subunits of Cannitoxin.** The \(\gamma\) and \(\beta_2\) subunits (100 nM) of cannitoxin had no significant effect on indirect twitches of the CBCNM preparation. However, the \(\alpha\) subunit (100 nM) caused a slight but significant inhibition of indirect twitches compared with the vehicle (Fig. 5a). None of the subunits had a significant effect on the response of the tissue to agonists (Fig. 5b).

**Phospholipase A₂ Activity**

Venom had a specific activity of 330 ± 5 μmol/min/mg, whereas that of the positive control (i.e., bee venom) was...
4-BPB significantly inhibited the PLA2 activity of cannitoxin (Table 2; \( n = 3–6 \)). The PLA2 activity of taipoxin, determined previously (Fohlman, 1979), is included for comparison. The \( \alpha \)-subunit seems to be the only subunit with a significant level of activity in cannitoxin and taipoxin (Table 2).

**Discussion**

This study describes the isolation and the pharmacological characterization of the first presynaptic neurotoxin, cannitoxin, from the venom of *O. scutellatus canni*. The venom from the Papuan taipan has postsynaptic neurotoxic activity (Crachi et al., 1999a) that is neutralized by the prior addition of taipan antivenom (Crachi et al., 1999b). However, in the current study, antivenom added at the \( t_{90} \) time point failed to reverse the indirect twitches, indicating the presence of a presynaptic neurotoxin(s). Such neurotoxins are unable to be reversed by the addition of antivenon once they have been bound and internalized (Fohlman et al., 1976) due to the physical damage to the presynaptic membrane by phospholipid hydrolysis.

The molecular mass of cannitoxin was determined as 45,000 Da using a series of known molecular weight standards on nondenaturing media. RP-HPLC of cannitoxin produced four main peaks, the molecular masses of

![Fig. 4](https://example.com/fig4.png)

**Fig. 4.** Effect of cannitoxin (11–66 nM) alone and cannitoxin in the presence of taipan antivenom, 4-BPB, or vehicle (i.e., acetone) on indirect twitches (a) and response to exogenous agonists (b). \(*, P < 0.05, \text{ significantly different compared with cannitoxin in the presence of antivenom; one-way ANOVA.}^{*}\) \( n = 4 \).

![Fig. 5](https://example.com/fig5.png)

**Fig. 5.** Effect of \( \alpha, \beta, \) and \( \gamma \) subunits (100 nM) and vehicle (i.e., 0.1% BSA) on nerve-mediated twitches (a) and response to exogenous agonists (b) of the CBCNM preparation. \(*, P < 0.05, \text{ significantly different compared with vehicle; one-way ANOVA; } n = 3–6 \).
TABLE 2

<table>
<thead>
<tr>
<th>PL\textsubscript{A}2 activities</th>
<th>PL\textsubscript{A}2 Activity (\textmu mol/min/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taipoxin</td>
<td>0.4*</td>
</tr>
<tr>
<td>(\alpha)-Taipoxin</td>
<td>3.8*</td>
</tr>
<tr>
<td>(\beta)-Taipoxin</td>
<td>0*</td>
</tr>
<tr>
<td>(\gamma)-Taipoxin</td>
<td>0.7*</td>
</tr>
<tr>
<td>Cannitoxin</td>
<td>90.5 \pm 1.1</td>
</tr>
<tr>
<td>Cannitoxin + 4-BPB</td>
<td>2.1 \pm 0.1</td>
</tr>
<tr>
<td>(\alpha)-Cannitoxin</td>
<td>129.7 \pm 12.4</td>
</tr>
<tr>
<td>(\beta)-Cannitoxin</td>
<td>4.8 \pm 3.8</td>
</tr>
<tr>
<td>(\gamma)-Cannitoxin</td>
<td>23.4 \pm 7.6</td>
</tr>
</tbody>
</table>

*Data from Fohlman et al. (1979); it should be noted that the activity of taipoxin and its subunits were obtained using different assays, so caution should be exercised when comparing activity with cannitoxin.

PL\textsubscript{A}2 enzymes are found in the venoms of snakes of all families (Harris, 1991); hence, the whole venom, cannitoxin, and its subunits were examined for PL\textsubscript{A}2 activity. The level of activity displayed by cannitoxin was approximately 3-fold less than that of the whole venom, suggesting the presence of other venom components with PL\textsubscript{A}2 activity. Although there is no quantitative relationship between the potency and PL\textsubscript{A}2 activity of presynaptic neurotoxins, inhibition of enzymatic activity is known to prevent their toxic effects (Yang, 1997). Thus, to test whether the PL\textsubscript{A}2 activity of cannitoxin is essential for its toxic effects, it was subjected to 4-BPB modification. Previous studies have shown that PL\textsubscript{A}2 activity can be inhibited by selective acylation of His48 residue using 4-BPB (Volwerk et al., 1974; Abe et al., 1977). When cannitoxin was incubated with 4-BPB, PL\textsubscript{A}2 activity as well as the neurotoxic effects was abolished. This suggests that PL\textsubscript{A}2 activity is essential for the neurotoxic effects of cannitoxin. Similarly, modification by 4-BPB inhibits the effects of other presynaptic neurotoxins such as taipoxin (Fohlman et al., 1979) and \(\beta\)-bungarotoxin (Abe et al., 1977). Further similarity between cannitoxin and taipoxin is highlighted by the PL\textsubscript{A}2 activity being largely confined to the \(\alpha\)-subunit of cannitoxin.

Given the medical importance of this species, it was important to assess the efficacy of the commercially available taipan antivenom in neutralizing the effects of cannitoxin. Prior addition of antivenom prevented the toxin-induced inhibition of indirect twitches in the CBCNM preparation. Addition of antivenom after the addition of toxin was not undertaken in this study since a similar experiment involving whole venom confirmed the irreversible nature of presynaptic neurotoxins.

Presynaptic neurotoxins with PL\textsubscript{A}2 activity can be classified based on structure as being single, dimeric, or multichain complexes (Yang, 1997). Multichain neurotoxins consist of several different polypeptide chains held together by noncovalent interactions, with at least one of the subunits having toxic activity on its own (Yang, 1997). However, previous studies have shown that the activity of the toxic subunit is far less in comparison with the native toxin (Fohlman et al., 1976; Francis et al., 1993). Therefore, the subunits of cannitoxin were tested for their neurotoxic effects. Despite having a high level of PL\textsubscript{A}2 activity, only the \(\alpha\) subunit at a much higher concentration (100 nM) caused a slight but statistically significant decrease in the indirect twitches. This further proves the lack of any quantitative relationship between enzymatic and toxic activity as well as the possibility of the other subunits acting as chaperones (Fohlman et al., 1976). The toxic effects and PL\textsubscript{A}2 activity of the \(\beta\) isosubunit could not be tested because of its low abundance in the venom and hence the small amount isolated. However, because the \(\beta\) and \(\beta_2\) subunits of cannitoxin are isomers and given the high sequence identity between \(\beta_1\) taipoxin and \(\beta\) cannitoxin, it is unlikely that the latter subunit has any neurotoxic activity. Neither \(\beta\) subunit of taipoxin is known to have toxic activity (Fohlman et al., 1976).

In conclusion, cannitoxin is the first neurotoxin to be isolated from the venom of the Papuan taipan. Cannitoxin is a presynaptic neurotoxin consisting of three different subunits held together by noncovalent interactions. The toxic effects of cannitoxin can be neutralized by commercially available CTPV and are dependent on its PL\textsubscript{A}2 activity. Cannitoxin is a major neurotoxic component of the venom; thus, it is likely to be responsible for much of the neurotoxic effect observed in envenomed patients.

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