ω-Conotoxin CVID Inhibits a Pharmacologically Distinct Voltage-Sensitive Calcium Channel Associated with Transmitter Release from Preganglionic Nerve Terminals

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Neurotransmitter release from preganglionic parasympathetic neurons is resistant to inhibition by selective antagonists of L-, N-, P/Q-, R-, and T-type calcium channels. In this study, the effects of different ω-conotoxins from genus Conus were investigated on current flow-through cloned voltage-sensitive calcium channels expressed in Xenopus oocytes and nerve-evoked transmitter release from the intact preganglionic cholinergic nerve innervating the rat submandibular ganglia. Our results indicate that ω-conotoxin CVID from Conus catus inhibits a pharmacologically distinct voltage-sensitive calcium channel involved in neurotransmitter release, whereas ω-conotoxin MVIIA had no effect. ω-Conotoxin CVID and MVIIA inhibited depolarization-activated Ba2+ currents recorded from oocytes expressing N-type but not L- or R-type calcium channels. High affinity inhibition of the CVID-sensitive calcium channel was enhanced when position 10 of the ω-conotoxin was occupied by the smaller residue lysine as found in CVID instead of an arginine as found in MVIIA. Given that relatively small differences in the sequence of the N-type calcium channel α1 subunit can influence ω-conotoxin access (Feng, Z. P., Hamid, J., Doering, C., Bosey, G. M., Snutch, T. P., and Zamponi, G. W. (2001) J. Biol. Chem. 276, 15728–15735), it is likely that the calcium channel in preganglionic nerve terminals targeted by CVID is a N-type (Ca2,2) calcium channel variant.

Venom of the predatory marine gastropods of the genus Conus (cone snails) contain a unique array of peptides whose pharmacological potential remains largely unexploited (1). These peptides have been classified based on their pharmacological target and structure (2, 3). One important class, the ω-conotoxins, utilizes a four-loop framework to selectively inhibit N-type voltage-sensitive calcium channels (VSCCs) found in the central and peripheral nervous systems of vertebrates (4).

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¶ The abbreviations used are: VSCC, voltage-sensitive calcium channel; EPSP, excitatory postsynaptic potential; HFTU, 2-(1H-benzotriazol-1-yl)-1,3,3,2-tetramethyluronium hexafluorophosphate; BAPTA, 1,2-bis(O-aminophenoxyl)ethane-N,N,N’,N’-tetraacetate.

Neurotransmitter release from preganglionic parasympathetic neurons has been found to be resistant to inhibition by a range of selective calcium channel antagonists of L-, N-, P/Q-, R-, and T-type calcium channels (5–8). A recently discovered ω-conotoxin from Conus catus (CVID) is highly selective for N-type over P/Q-type VSCCs (9) and shows potent analgesic activity in rats (10). In this study, we investigated the effects of CVID on autonomic neurotransmission using conventional intracellular microelectrode recording techniques. ω-Conotoxin CVID was found to be a potent inhibitor of neurally evoked transmitter release from the intact preganglionic cholinergic nerves innervating the rat submandibular ganglia, whereas ω-conotoxin MVIIA had no effect.

The orientation and nature of the residues in loop 2 of ω-conotoxins have been shown to be crucial for selective binding to the N-type VSCC (11–15). Since the only sequence difference in loop 2 between CVID and MVIIA is at position 10, we investigated the importance of this position for ω-conotoxin structure and ability to block neurotransmitter release from preganglionic parasympathetic neurons. The inhibition of preganglionic transmitter release was favored in ω-conotoxin with a Lys at position 10.

EXPERIMENTAL PROCEDURES

Submandibular Ganglia Preparation—2–5-week-old rats were anesthetized and killed by cervical fracture prior to the removal of the submandibular ganglia in accordance with the guidelines of the University of Queensland Animal Experimentation Committee. The submandibular ganglia were removed as described previously (8, 16). Individual preparations were pinned to the Sylgard (Dow Corning) covered base of a 2-ml Perspex organ bath. Preparations were continuously perfused at a rate of 2 ml/min with a Krebs phosphate solution of the following composition (in mM): 118.4 NaCl, 25.0 NaHCO3, 1.13 NaH2PO4, 1.8 CaCl2, 4.7 KCl, 1.3 MgCl2, and 11.1 glucose, gassed with a mixture of 95% O2 and 5% CO2 to pH 7.4, and maintained at 36–37 °C.

Intracellular Recordings and Analysis—The lingual nerve was field-stimulated by voltage pulses via bare platinum wires delivered from a digital stimulator (Pulsar 7, Frederick Haer & Company, Brunswick, ME) coupled to an optically isolated stimulation unit (Model D52, Digitimer Ltd., Welwyn Garden City, United Kingdom). Intracellular recordings were made from individual ganglion cells using glass microelectrodes filled with 5 M potassium acetate (resistances 70–120 MΩ) coupled to an Axoclamp-2A amplifier (Axon Instruments Inc., Union City, CA) in bridge mode and stored on a digital audio tape using a digital tape recorder (DTR-1204, BioLogic Science Instruments, Claix, France). Evoked events were digitized at 5–10 kHz and transferred to a computer using an analogue-to-digital converter (Digitdata 1200A interface) and Axograph software (Axon Instruments Inc.). The amplitude, frequency, rise time, and latency of evoked and spontaneous events were analyzed using Axograph 2 (Axon Instruments Inc.). The mean resting membrane potential of the sub-
mandibular ganglion neurons was $-63.8 \pm 0.6$ mV ($n = 52$). The mean base line was determined by averaging the initial part of the digitized signal between the stimulus artifact and the onset of the response. Data are expressed as the mean $\pm$ S.E., and $n$ values refer to the number of preparations. Data were analyzed statistically using Student's paired $t$ test with the level of significance being taken as $p < 0.05$.

Electrophysiological Recording of Recombinant Ca$^{2+}$ Channel Currents in Xenopus Oocytes—Capped RNA transcripts encoding full-length rat $\alpha_{1B}$ and $\beta_2$ (a gift of D. Lipscombe, Brown University) and rabbit $\alpha_{1C}$ and $\beta_3$ (a gift of G. Zamponi, University of Calgary) were synthesized using a mMESSAGE mMACHINE in vitro transcription kit (Ambion). Xenopus laevis stage V–VI oocytes were removed and treated with collagenase (Sigma type I) for defolliculation. For N- or L-type Ca$^{2+}$ channel expression, the oocytes were then injected with cRNA mixtures of either $\alpha_{1C}$ (2.5 ng/cell) or $\alpha_{1B}$ (5 ng/cell) in combination with $\alpha_2\delta$ and $\beta_2$ (a gift of G. Zamponi, University of Calgary) was injected intranuclearly in combination with the cRNA injection of $\alpha_2\delta$ and $\beta_2$ (2.5 ng/cell each). The oocytes were incubated at 18°C in NDB96 solution (in mM): 96 NaCl, 2 KCl, 1 CaCl$_2$, 1 MgCl$_2$, 5 HEPES, 5 pyruvic acid, and 50 µg/ml gentamicin, pH 7.5, prior to recording. 3–8 days after cRNA/cDNA injection, whole cell Ca$^{2+}$ channel currents were recorded from oocytes using the two-electrode (virtual ground circuit) voltage clamp technique. Microelectrodes were filled with 3 M KCl and typically had resistances of 0.3–1.5 megohms. All recordings were made at room temperature (20–23°C) using bath solution containing the following components (in mM): 5 BaCl$_2$, 85 tetraethylammonium hydroxide, 5 KCl, and 5 HEPES titrated to pH 7.4 with methanesulfonic acid. During recording, oocytes were perfused continuously at a rate of $\sim 1.5$ ml/min. The activation of Cl$^-$ current by Ba$^{2+}$ influx through the Ca$^{2+}$ channel was eliminated by injecting 50 nl of 50 mM BAPTA-Na$_2$ at least 15 min prior to recording. Using a GeneClamp 500B amplifier and pCLAMP 8 software (Axon Instruments Inc.), data were acquired at 10 kHz after low pass filtration at 1 kHz and leak-subtracted on-line using a P/4 protocol and analyzed. Currents were evoked with 100-ms depolarizing pulses to 0 mV from a holding potential of $\sim 80$ mV at 20-s intervals. Peak current was measured before and during the perfusion of $\omega$-conotoxins and antagonists for up to 10 min.

Radioisotopic Binding—Preparation of rat brain membrane and [$^{125}$I]GVIA and radioligand displacement protocols were as described previously (9, 14, 15). $\omega$-Conotoxin Synthesis—$\omega$-Conotoxin CVID, [K10R]CVID, MVIIA, [R10K]MVIIA, and [Y13F]MVIIA were assembled by manual stepwise synthesis using in situ neutralization with Boc chemistry (19). Starting from methylbenzhydrylamine HCl resin (0.5-mmol scale), 4 eq of Boc-protected amino acids were coupled to the growing chain using 4 eq of 0.5 M HBTU in N,N-dimethylformamide and 5 eq of N,N-diisopropyl-ethylamine. The side chain protection chosen was Arg(Tos), Aasp(O-CHex), Lys(CIZ), Thr(Bzl), Tyr(BrZ), Ser(Bzl), and Cys(p-MeBzl). The chain assembly was monitored after each step by the quantitative ninhydrin method and double-coupled when coupling yields were <99.7%. The capping of the remaining amino groups by acetylation was

**FIG. 1.** Effect of $\omega$-conotoxin CVID on nerve-evoked EPSPs and spontaneous EPSPs in the rat submandibular ganglion. A, control EPSPs evoked by trains of 10 stimuli at 1 Hz. B, postsynaptic responses evoked 20 min after the bath application of 30 nm CVID. C, postsynaptic responses evoked 20 min after the bath application of 300 nm CVID. Inset, effect of CVID (100 nm) on spontaneous EPSPs. D, dose-response relationship obtained for the effects of the calcium channel antagonists, $\omega$-conotoxin CVID, $\omega$-conotoxin MVIIA, $\omega$-grammotoxin SIA, and $\omega$-agatoxin IVA on EPSP peak amplitude. The solid lines represent the best fit of the data for each toxin.

**FIG. 2.** Effect of [K10R]CVID, $\omega$-conotoxin MVIIA, and [R10K]MVIIA on EPSPs in the rat submandibular ganglion. EPSPs were evoked by trains of 10 stimuli at 0.5 Hz. A, 10 superimposed traces in the absence (control) and presence of 100 nm [K10R]CVID. B, 10 superimposed traces in the absence (control) and presence of 100 nm 1MVIIA. C, 10 superimposed traces in the absence (control) and presence of 100 nm [R10K]MVIIA. D, histogram of the percentage inhibition of EPSP amplitude by equivalent concentrations (100 nm) of CVID, [K10R]CVID, MVIIA, and [R10K]MVIIA.
ω-Conotoxin Inhibition of Resistant Transmitter Release

Table I

| Peptide | Sequence | pIC50 (95% CI) |
|---------|----------|-------------|
| CVID    | CKSKGAKSK1YMDCCTGGSCSGTVGRC<sup>b</sup> | 10.42 (±0.10) |
| [R10R] CVID | CKSKGAKSK1YMDCCTGGSCSGTVGRC<sup>b</sup> | 10.81 (±0.13) |
| [Y10F] CVID | CKSKGAKSK1YMDCCTGGSCSGTVGRC<sup>b</sup> | 7.79 (±0.042) |
| MVIA    | CKGKRSGKMDDCTGGCRS–GKGC<sup>b</sup> | 10.65 (±0.16) |
| [R10K] MVIA | CKGKRSGKMDDCTGGCRS–GKGC<sup>b</sup> | 10.62 (±0.046) |
| GVIA    | CKSOGS2SC0SYNCNGRSCN0YTIRKC<sup>b</sup> | 10.63 (±0.17) |

* Potency presented as the pIC₅₀ (−logIC₅₀) for displacement of [¹²⁵I]GVIA from rat brain membrane.

* Amidated C terminus.

Table II

| VSCC antagonist | Transmitter release | α₂₅ (P/Q type) | α₁₈ (N-type) | α₁₆ (L-type) | α₃₂ (R-type) |
|-----------------|---------------------|----------------|-------------|-------------|-------------|
| ω-CVID          | + (n = 13)          | – (9)          | + (n = 5) (9) | – (n = 3)   | – (n = 4)   |
| ω-MVIA          | – (n = 5)           | – (9)          | + (n = 5) (9, 31) | – (n = 3)   | – (n = 4)   |
| ω-GVIA          | – (n = 9)           | – (29)         | + (30, 31)   | – (32)      | – (33)      |
| ω-MVIIIC        | – (n = 5)           | + (29, 30)     | + (30)       | ?           | – (34)      |
| ω-agatoxin IVA  | – (n = 4)           | + (29, 30)     | + (30)       | – (32)      | + (33, 34)  |
| ω-granatoxin SIA| – (n = 8)           | + (30)         | + (28, 30)   | ?           | – (28)      |
| SNX-482         | – (n = 4)           | – (35)         | – (28, 35)   | + (28)      | + (28, 35)  |
| Nifedipine      | – (n = 8)           | – (36)         | – (n = 3) (31, 36) | + (32, 36) | – (36)      |

Evidence for VSCC inhibition (+), no inhibition (−), or unknown (?) are indicated. Numbers of replicate experiments (n) in the present study or reference numbers are shown.

RESULTS

Characteristics of Excitatory Postsynaptic Potentials (EPSPs)—Stimulation of the lingual nerve with trains of stimuli (0.1–10 Hz, 4–50 V, pulse-width 0.05–0.25 ms) evoked EPSPs, which could initiate action potentials in the cell bodies of the postsynaptic neurons of the rat submandibular ganglion. The postsynaptic neurons have been classified into three types by their responses to these trains of stimuli (8). In this study, neurons in which supramaximal stimulation of the preganglionic nerve fibers at 0.1 Hz either evoked a suprathreshold EPSP and action potential in response to every stimulus (strong input synapse, ≥50% of total number of neurons) (Fig. 1A) or where the EPSP does not usually reach threshold for the initiation of an action potential (weak input synapse, −25% of neurons studied) were used. EPSPs recorded from neurons receiving either strong or weak inputs were abolished by hexamethonium (100 μM) and mecamylamine (10 μM) indicating that EPSPs were mediated by acetylcholine acting at nicotinic receptors.

Effects of Calcium Channel Inhibitors on EPSPs—ω-Conotoxin CVID produced a reversible and concentration-dependent inhibition of EPSPs (Fig. 1). Control EPSPs from both strong and weak input neurons had a mean amplitude of 26.2 ± 2.5 mV, and 20 min after bath application of CVID (100 nM), these EPSPs were reduced by 66% to a mean amplitude of 9.1 mV, and 20 min after bath application of CVID (100 nM), these trains of stimuli (8). In this study, neurons in which supramaximal stimulation of the preganglionic nerve fibers at 0.1 Hz either evoked a suprathreshold EPSP and action potential in response to every stimulus (strong input synapse, ≥50% of total number of neurons) (Fig. 1A) or where the EPSP does not usually reach threshold for the initiation of an action potential (weak input synapse, −25% of neurons studied) were used. EPSPs recorded from neurons receiving either strong or weak inputs were abolished by hexamethonium (100 μM) and mecamylamine (10 μM) indicating that EPSPs were mediated by acetylcholine acting at nicotinic receptors.

Drugs—Drugs were dissolved in the Krebs phosphate solution preceding the preparation, and the effects were evaluated after they reached equilibrium (≥20 min). The dose-response relations were determined for ω-conotoxins, whereas other drugs were bath-applied at a maximally effective concentration. In addition to the synthesized ω-conotoxins, we tested ω-agatoxin IVA, ω-conotoxin GVIA, ω-conotoxin MVIIIC (Alamone Laboratories, Jerusalem, Israel), SNX-482 (Peptide Institute Inc., Osaka, Japan), cadmium chloride, hexamethonium, mecamylamine, nifedipine, and tetrodotoxin (Sigma). ω-Granatoxin SIA was a kind gift from Dr. Rick Lampe (Zenea Pharmaceuticals, Wilmington, DE).

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The effect of CVID contrasts with the effects of 300 nM ω-conotoxin GVIA (N-type), 100 nM ω-agatoxin IVA, 300 nM ω-conotoxin MVIIIC (P/Q-type), 300 nM ω-granatoxin SIA, 300 nM SNX-482 (R-type), and 30 μM nifedipine (L-type), which had little or no inhibitory effect on evoked and spontaneous transmitter release in rat submandibular ganglia either alone or in combination with each other (n = 3–13 per drug) (Fig. 1D).
confirming previous results (8). In a proportion of weak input neurons, GVIA inhibited EPSPs by up to 12% of the control amplitude (5 of 8 preparations) (Fig. 1D) with an IC_{50} of 60 nM, suggesting that GVIA-sensitive calcium channels play a minor role in neurotransmission in the rat submandibular ganglia. GVIA had no effect on strong input neurons (n = 4). EPSPs were consistently abolished by low concentrations of the non-specific calcium channel blocker Cd^{2+} (30 μM, n = 8), indicating that neurally evoked transmitter release from preganglionic neurons was dependent on calcium influx through VSCCs.

Of the characterized ω-conotoxins, CVID most closely resembles MVIIA in structure. Despite this structural similarity, MVIIA (10–1000 nM) failed to produce detectable inhibition of EPSPs (n = 4) (Fig. 2B). Since loop 2 contributes mostly to ω-conotoxin potency at N-type VSCCs, we investigated the influence of a Lys/Arg swap at position 10 in CVID and MVIIA was dose-dependent and slowly reversed upon washout. In comparison to MVIIA, [K10R]CVID and [R10K]MVIIA were examined on L-, N-, and R-type Ca^{2+} channels (see Table II) (29). The inhibitory effect of [R10K]MVIIA at 293 K in 95% H_{2}O, 5% D_{2}O. Residue numbers are labeled according to the CVID sequence. Three-dimensional structures of CVID, MVIIA, and [R10K]MVIIA (Fig. 3A) were superimposed with an IC_{50} of 60 nM, confirming that the hydroxyl group in Tyr-13 of CVID has a similar influence on the transmitter release recorded from oocytes expressing N-type, R-type Ca^{2+} channels in combination with α_{δ} and β_{γ} using 5 mM Ba^{2+} as the charge carrier. Bath application of either ω-conotoxins CVID or MVIIA (300 nM) inhibited the depolarization-activated inward Ba^{2+} current recorded from oocytes expressing N-type but had no effect on current through L- or R-type Ca^{2+} channels (see Table II) (29–36). The inhibition of Ba^{2+} currents through N-type Ca^{2+} channels by ω-conotoxin CVID and MVIIA was dose-dependent and slowly reversed upon washout.

Three Dimensional Structures—Ha chemical shifts relative to random coil values are a sensitive measurement of backbone conformation for disulfide-bonded isomers and can be used to locate local conformational differences across structurally related peptides (25, 26). Ha chemical shifts were similar for [K10R]CVID and [R10K]MVIIA compared with the native peptides CVID and MVIIA (Fig. 3A), indicating that any global structural changes on swapping residue 10 are minimal. The three-dimensional structure of [R10K]MVIIA differed from MVIIA by a root mean square deviation of 0.461 across the heavy backbone atoms, confirming the absence of any significant structural change. This is seen in the ribbon diagram showing the superimposition of the 17 lowest energy conformations of the side chains at positions 10 (Lys or Arg) and 13 (Tyr) are also shown on the right side. On the right side are shown the ribbon diagrams rotated 180° around the y axis and the calculated Connolly surface area of Lys/Arg-10 and the orientation of Tyr-13 in loop 2 (residues 8–14 are shown). Structures of the lowest energy conformation were superimposed over backbone atoms Ca, C, and N (1–27 and 1–25) for CVID, MVIIA, and [R10K]MVIIA, respectively (Insight II 2000).
surface area for residue 10 over the 17–20 lowest energy conformations in Insight II. The rank order of the solvent-accessible surface area was MVIIA > [R10K]MVIIA > CVID (156 ± 8.0, 147 ± 8.1, and 92 ± 4.6 Å², respectively). A complete three-dimensional structure was not calculated for [K10R]CVID, because it had the same potency at the N-type VSCC and conserved Ha chemical shift differences compared with CVID especially across loop 2 (Fig. 3A).

DISCUSSION

Neurotransmitter release from the preganglionic cholinergic neurons innervating the rat submandibular ganglia is resistant to inhibition by commonly used selective antagonists of N-, L-, P/Q-, R-, and T-type calcium channels (8, 17). The aim of this study was to investigate the effects of the recently identified ω-conotoxin CVID, the most selective N-type versus P/Q-type VSCC blocker, on transmitter release from these nerve terminals. CVID (IC_<sub>50</sub> = 32 nM) produced up to 80% inhibition of nerve-evoked transmitter release without affecting spontaneous transmitter release, and this inhibition was reversed upon washout. In contrast, nerve-evoked transmitter release was unaffected by MVIIA (≤1 μM). Interestingly, the potency of CVID to inhibit transmitter release from preganglionic cholinergic neurons was similar to the potency of both CVID and MVIIA to inhibit N-type VSCCs in postganglionic sympathetic neurons of rat vas deferens (9).

Loop 2 of ω-conotoxins has been identified as the primary loop involved in binding to the N-type VSCC (11, 13, 25) with the hydroxyl group on Tyr-13 on both ω-conotoxin GVIA and MVIIA found to be a critical determinant of high interaction at the N-type VSCC (12, 27). The 400-fold reduction in potency of [Y13F]CVID for rat brain N-type VSCC confirmed a key role of Tyr-13 in CVID. [Y13F]CVID was also inactive on the EPSPs, indicating that N-type and CVID-sensitive VSCCs both require the hydroxyl of Tyr-13. One possible conclusion from this result is that CVID may bind to both VSCCs in a similar manner. Because loop 2 of MVIIA and CVID vary only at position 10 (Lys or Arg, respectively) (see Table 1), we investigated the role of this position contributing to the selectivity differences observed between MVIIA and CVID by swapping these residues. [K10R]CVID (100 nM) produced significantly less (≤20% inhibition of transmitter release than CVID, indicating that Lys-10 was the preferred residue for resistant-type VSCC inhibition by CVID. Supporting this conclusion, Lys-10 was found to markedly enhance MVIIA inhibition of resistant-type VSCCs with the [R10K]MVIIA (100 nM) causing 47% inhibition of evoked transmitter release (i.e. >100-fold more than MVIIA). None of the ω-conotoxins investigated affected spontaneous transmitter release or the resting membrane potential of the post synaptic cell, indicating a selective action at presynaptic VSCCs.

The effects of the L-, N-, P/Q-, and R-type VSCC antagonists (nifedipine, ω-conotoxin GVIA, ω-conotoxin MVIIIC, ω-agatoxin IVA, ω-gammatomotoxin SIA, and SNX-482) were investigated on the transmitter release remaining after the application of CVID. None of these VSCC inhibitors, either applied alone or in combination, had any effect on the CVID-resistant neurotransmitter release. However, the CVID-resistant release was reversibly abolished by Cd²⁺, indicating its dependence on calcium entry into nerve terminals through VSCCs. Because the R-type VSCC inhibitors used do not inhibit all R-type VSCCs (28), we investigated the effects of ω-conotoxins CVID and MVIIA on current-flow through cloned L- or R-type calcium channels expressed in Xenopus oocytes. Both CVID and MVIIA had no effect on depolarization-activated Ba²⁺ currents through L- or R-type calcium channels but potently inhibited current through N-type VSCCs (see Table II) as reported previously (9).

In an attempt to understand the structural basis of the selectivity differences between ω-conotoxin MVIIA and [R10K]MVIIA at the CVID-sensitive VSCCs, we determined their secondary and tertiary structures by ¹H NMR. From these studies, it was evident that residue replacements at position 10 had little effect on the global fold or structure of CVID or MVIIA. However, examining the solvent-accessible surface area at position 10 revealed a trend of decreasing surface area that correlated with activity at the CVID-sensitive VSCCs in preganglionic neurons. It is possible that the degree of exposure may influence the selectivity of ω-conotoxins with the most exposed residue, Arg in MVIIA, creating a clash that precludes binding to certain VSCCs. Recently, Feng et al. (37) identified that a single residue change on an extracellular loop of the N-type VSCC (G1326) can influence ω-conotoxin access. Thus, relatively small differences in N-type VSCC sequence may significantly alter ω-conotoxin affinity, raising to the possibility that the VSCC targeted by CVID in preganglionic nerve terminals may be a N-type (Ca₂,2) calcium channel variant that does not bind all ω-conotoxins. Two functionally distinct variants of the α₁B subunit, α₁B₄ and α₁B₅ of the N-type calcium channel that differ at two loci (four amino acids (SFMG) in IIS3–S4 and two amino acids (Glu/Thr) in IVS3–S4) have been shown to be reciprocally expressed in rat brain and sympathetic ganglia (38). The selective expression of pharmacologically distinct VSCC splice variants of α₁B subunits in different regions of the nervous system is proposed to underlie the differential block of preganglionic nerve terminal VSCCs by N-type selective ω-conotoxins. Alternative splicing of the domain II–III linker region of the human N-type (Ca₂,2) calcium channel α₁B subunit has recently been shown to differentially affect ω-conotoxin MVIIA and GVIA block of the calcium channel current (39).

In conclusion, CVID was found to be a potent inhibitor of the pharmacologically distinct VSCC controlling a major component of transmitter release in preganglionic cholinergic nerve terminals. CVID may help to define the nature and role of this VSCC. Position 10 appears to play a key role in influencing ω-conotoxin affinity for this N-type-related VSCC. Given that [¹²⁵I]CVID labels fewer sites in rat brain than [¹²⁵I]MVIIA (9), it is unlikely that these resistant-type VSCCs are among the high voltage-activated VSCCs labeled by [¹²⁵I]Aga-IIIA in rat brain (40). Intrathecal CVID (AM336) and MVIIA (Ziconotide) are currently being evaluated clinically as treatments for severe pain (10, 41). Because parasympathetic nerves arise from spinal cord neurons, it is possible that CVID-sensitive VSCCs are also expressed and play a role in neurotransmission in the spinal cord. Given the differences in the selectivity of ω-conotoxin CVID and MVIIA for this VSCC subtype, these ω-conotoxins may have different profiles of activity in the central (for review see Refs. 10 and 42) as well as the peripheral nervous system.

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