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CONOTOXIN'S PHARMACOLOGICAL CHARACTERIZATION AND APPLICATIONS: A REVIEW

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Abstract

Conotoxins from marine cone snails are small, disulfide-rich peptides that act with specificity on various receptors and channels, making these powerful probes to study the properties of voltage and ligand-gated ion channels. Conotoxin drug candidates have shown remarkable therapeutic promise in managing pain, addiction, and neurodegenerative disorders. A systematic review following th e PRISMA guidelines was conducted to assess the pharmacological effects of conotoxins on various channels and receptors and the conotoxins' effects in broader in vivo and in vitro biological assays. PubMed and Google Scholar articles assessing the ability of conotoxins to inhibit receptors and channels and the conotoxins-induced biological response were eligible for inclusion. Twenty-seven studies met the inclusion criteria. Studies of alpha conotoxin reported the peptide as an excellent tool for distinguishing neuronal and muscle nAChRs

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and have a high potency of blocking nAChRs even at nanomolar concentrations. While μ-conotoxin isolated from piscivorous *Conus* potently blocks sodium channels, with μ-GIIIA and μ-GIIIB abolishing the twitch response of the muscle. δ-GmVIA slows down the sodium-current inactivation, while δ-TxVIA does not. ω-TxVII significantly and reversibly blocked the L-type Ca2+ current. However, all the evidence reported in this review was obtained from studies with a serious risk of bias due to a lack of blind outcome assessment, thereby limiting the strength of the pieces of evidence. Blinded RCTs may improve confidence in concluding the biological effects of various conotoxins.

Index Terms: calcium channel, Conotoxin, *Conus*, nicotinic acetylcholine receptor, PRISMA, pharmacology, sodium channel

1. INTRODUCTION

It is well recognized that the opioid crisis is one of the most significant and recent severe public health issues, with more than 93,000 Americans dying from drug overdose between December 2019 and December 2020 [1]. Hence, it is pivotal to examine treatment and recovery services, strengthen the understanding regarding pain and addiction, and advance evidence-based medical practices for pain management. A review of the opioid crisis has shown that an interprofessional approach is required for patients prescribed opioids [2]. Healthcare providers play a significant role in appropriate pain management education and prescription. Researchers are also essential in providing and strengthening the understanding of pain management and advancing treatments and practices.

The venom of marine *Conus* contains small, disulfide-rich peptides that act with excellent specificity on various channels and receptors. α-conotoxin is an antagonist of the nicotinic acetylcholine receptors (nAChRs). δ-conotoxin slows down the inactivation of the voltage- gated sodium channels, while μ-conotoxins block the same sodium channels. In addition, ω-conotoxins selectively inhibit calcium voltage- gated channels. Hence, these highly selective peptides can also be used as specific probes to study the structure-function relationships of channels and receptors; similarly, conotoxins represent potential therapeutic candidates for alleviating pain and aiding the functional recovery of neurons. *Conus*-derived therapeutics include Ziconotide, an intrathecal non-opioid analgesic drug derived from ω-conotoxin MVIIA of *Conus* magus approved by the U.S. Food and Drug Administration under the name Prialt and used for treating intractable pain. Several other conopeptides are undergoing clinical trials and assessments for possible therapeutic applications.

This systematic review presents reported conotoxins' mechanisms of action on various receptors and channels and conotoxins-induced biological activity to gain a deeper understanding of conotoxins' specificity and inhibition kinetics, as well as their potential analgesic effect and pharmacological application.

2. METHODOLOGY

A systematic review was performed following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The associated questions regarding the pharmacological application of conotoxins were formulated using the patient population,

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intervention, comparison, and outcomes (PICOs) framework to summarize the *alpha, mu, delta*, and *omega* conotoxins' from the selected *Conus* species (1) effects on targeted receptors and ion channels; (2) the action mechanisms to its target receptor, and (3) the biological activities and hazards induced.

2.1 Inclusion and Exclusion Criteria

Original articles assessing the effects of alpha (α) and mu (μ) conotoxin from the piscivorous *Conus* species *C. catus*, *C. geographus*, *C. tulipa*,

C. bullatus, and *C. magus* and omega (ω) and delta (δ) conotoxin from molluscivorous species *C. bandanus*, *C. marmoreus*, *C. textile*, *C. aulicus*, and *C. gloriamaris* on various receptors and ion channels. Studies that used 0.001-100 μM of native or synthesized conotoxins, clinical trials assessing the pharmacological effects or the broader biological roles of conotoxins on specific receptors and muscles, In vivo and In vitro studies with endpoints of assessing the effectiveness of blocking various receptors and ion channels of the native or synthesized conotoxins from the selected piscivorous and molluscivorous species, focusing on determining the mechanism of action of conotoxin on various muscles and receptors were eligible and included in the review. However, studies that are not in English, reviews, studies evaluating the purification and characterization of the conotoxin alone, assessing the chemical synthesis of conotoxin independently, and those that discuss the structural relationship of different conotoxins were excluded.

2.2 Literature Search Method

A systematic search was conducted on PubMed and Google Scholar for studies evaluating the effect of conotoxins on various channels and receptors from inception to September 2021 with relative key search terms of *Conus* species with mentioned corresponding conotoxins as mentioned. The search was duplicate-filtered and limited to English-published studies.

2.3 Study Selection and Data Extraction

The review authors independently reviewed titles and abstracts of studies retrieved through the search for their relevance and design. The papers were examined for eligibility and inclusion and counter verified. Extracted data was then cross-checked by another reviewer who has not participated in the extraction procedure. The bibliometric indices and population characteristics were also retrieved.

2.4 Data Items

This systematic review was performed to collate and analyze the effect of the selected conotoxins on various receptors, channels, and muscles. The clinical questions in association with PICOs are as follows:

Participants: Studies were considered for inclusion if people and animals of any age were used to assess the effect of conotoxin on specific receptors and muscles. Any in vitro and in vivo samples used to assess the activity and specificity of inhibition kinetics of conotoxins on receptors, channels, and muscles are also eligible.

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Intervention: Studies were considered for inclusion if the selected conotoxins were used in In vitro and In vivo samples to assess the effect of conotoxins on specific receptors and on bioassays.

Comparison: Studies were considered for inclusion if the conotoxin is compared to any comparator intervention.

Outcome: The outcome did not form part of the selection process. The result was structured into primary (conotoxin-blocked targeted receptors and channels, percentage of blocking targeted receptors and channels, and conotoxin-induced biological response) and secondary outcomes (native and synthetic conotoxin effectiveness in blocking targeted receptors and channels and effectiveness compared to other conotoxins).

2.5 Risk of Bias in Individual Study Assessment

This systematic review included both randomized and non-randomized studies about the α and μ conotoxin of the piscivorous cone snails and the δ and ω conotoxin of the molluscivorous cone snails. The researchers exercised the Cochrane Collaboration's risk of an assessment tool for evaluating the risk of bias in randomized trials and the Risk of Bias in Non-randomized Studies – of Intervention (ROBINS – I) for non-randomized studies.

2.6 Data Synthesis

The author independently reviewed all papers. A narrative synthesis was conducted to evaluate the varied associated results. Due to the sheer high degree of heterogeneity, studies that used various statistical tools, laboratory devices, and subjective evaluations, a narrative synthesis was utilized to create a pooled summary of findings.

3. RESULTS

The result of the systematic search is summarized in Fig. 1. The systematic search identified 6,300 studies; after initial deduplication, 2,074 unique studies remained. After applying the inclusion and exclusion criteria, only twenty-seven (27) studies remained to be included in the systematic review.

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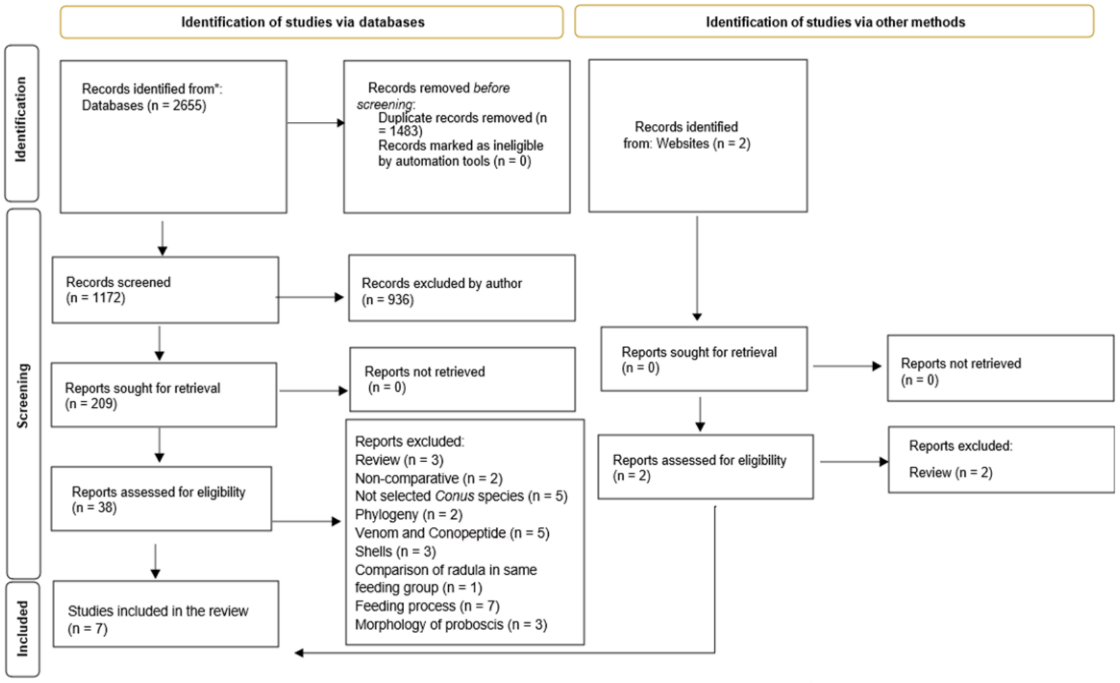

Fig 1: The PRISMA Flowchart

3.1 Included Studies

All studies provided level three evidence based on the Oxford Center for Evidence-Based Medicine 2011 scale. The twenty-seven (27) included studies used various samples to assess the conotoxin's ability to block and inhibit receptors and ion channels and their biological activities on various muscle types. Included studies utilized *Xenopus* oocytes expressing rat and mouse sodium channel subunits [3], [4], [5],[6],[7],[8]; a cell line modified to express sodium channel subunits [9]; *Xenopus* oocyte expressing nAChr subunits [10],[11],[12],[13], [14],[15],[16], 17]; *Aplysia* neurons [18],[19], rat ganglia neurons [20], 21]; and animal models including *Danio rerio*, *Mus musculus*, *Carassius auratus*, *Patella vulgate*, *Cornu aspersum*, *Lymnaea stagnalis*, *Gambusia affinis*, and *Rattus norvegicus* were used [10], [11], [13],[18],[20],[22],[23],[24],[25],[26],[27],[28].

3.2 Intervention

Twenty-seven (27) studies that meet this review's inclusion criteria were examined based on the conotoxin concentration-dependent blocking or inhibition of receptors and ion channels and their broader biological effects. The included studies utilized a variety of methods such as a static bath exposure of in vitro samples to conotoxins [3], [4], [5], [7], [8], [10], [29]; perfusion [9], [11], [12], [13], [16], [20], [21];

superfusion [6], [15], [20]; incubation after conotoxin application [12], [14], [15], [23,]; and other specific systems to apply the conotoxin

[17], [19], [22], [25]. Whereas *in vivo* studies that used animal models applied conotoxin through intramuscular [10], [13], [23], [27], [28],

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intracranial [18] [29], and intraperitoneal [11] [20] injection.

3.3 Outcomes

Of the twenty-seven (27) included studies, twenty-five (25) included the conotoxins' abilities and potencies in blocking or inhibiting a specific receptor or ion channel, while nine studies included the biological activity of conotoxin as outcomes.

3.4 Risk of Bias in Included Studies

The risk of bias assessment for the randomized trial is presented in Fig 2. The trial had a low risk of bias since the randomization of participants into groups was stated [9]. However, the performance and detection bias indicated a high risk of bias since assessors are not blinded. Fig. 3 presents the summary of the risk of bias assessment of all 26 non-randomized studies. All non-randomized studies were judged to be at serious risk of bias in the measurement of outcome data, due to a lack of blind outcome assessment.

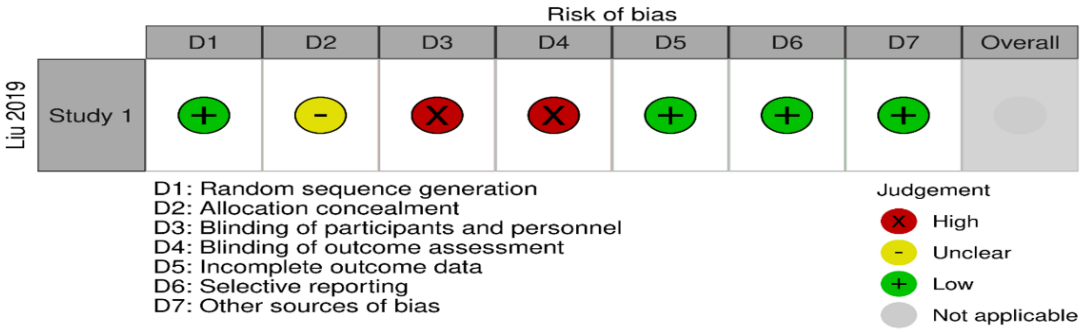

Fig 2: Risk of Bias was applied to each included study based on the review author's judgments regarding each risk of bias item

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Fig 3: Risk of Bias Summary: Review the authors' assessments of each risk of bias item for each included study

3.5 Primary Outcomes: Piscivorous alpha conotoxin

3.5.1 Conotoxin Inhibition and Block Percentage

The α-conotoxin of *C. geographus*, named GID, GIC, and GVIIIB are reported [12], [13], [15], [17]. α-GID has consistently antagonized nAChR receptors [15], [17]. The IC50 values of GID on α7 and α3β2 were 3 and 5 nM, respectively [15]. A similar study has recorded close results on the IC50 values of GID on α7, α3β2, and α4β2 at 5.1, 3.4, and

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128.6, respectively [17].

α-GIC does not block human muscle nAChR subtype but potently blocks ACh-induced response in human neuronal nAChRs (10 nM concentration producing a 91.5% block of ACh-induced response) that is reversed relatively rapidly in neuronal α3β2 subunits [15]. This finding suggests GIC has a high selectivity for α3β2 receptors compared to muscle nAChR subtypes. αS-GVIIIB caused little to no block in rat neuronal and human muscle nAChR subtypes. Treatment of 100 nM αS-GVIIIB caused ACh-induced blocking of 2.9%, α3β2; 7.7%, α6/α3β2β3; 6.5%, α3β4; 5.3%, α4β2; and 4.1% for α7. At 10-fold higher concentration, a partial block was observed in α3β2 and α6/α3β2β3 with ACh-induced responses of 48.3% and 54.0%, respectively [12].

The α-conotoxin of *C. magus*- MII has consistently been reported to antagonize nAChR receptors in a dose-dependent block on α3β2 receptors at nanomolar concentrations [11], [14], [21]. Native MII (3.7 nM – 222 nM) reduced the nACh-activated current response by 47-76%.

α-BuIA from *C. bullatus* [16], [20] reported its effects on nAChR subtypes [16] and calcium channels [20]. The venom potently blocks (α6/α4) β2β3 induced current by 92.6±2.6%, consistent with the IC_{50} = 0.43 nM [20]. In contrast, the binding affinity of BuIA and its analogs (cotx 2.1 and cotx 2.13) showed significantly enhanced affinity to human α7 nAChR; compared to novel conotoxin [16]. Additionally, BuIA and analogs induced calcium channel closure, leading to decreased calcium levels in dorsal root ganglion (DRG) neuron cells.

Studies assessing the blocking mechanism of CIA reported conotoxin's blocking of muscle type (α1)2δγβ1 nAChR with IC_{50} of 5.7 nM and the neuronal subtype α3β2 with >350-fold lower affinity (IC_{50} = 2.06μM), while no noticeable activity recorded on α7 and α4β2 subtypes even at higher concentrations (10 μM) [10]. Contrastingly, CIB blocked neuronal subtype α3β2 with an IC_{50} of 128.9 nM and α7 with an IC_{50} of 1.51 μM while CIB has no activity on muscle and α4β2 subtype at 10 μM.

These results suggest α-conotoxins potently block specific nAChR subtypes at nanomolar concentrations while effectively discriminating and distinguishing nAChR subunits. MII and GID potently block α3β2, and α7 nAChRs, BuIA potently blocks (α6/α4) β2β3, GIC potently blocks neuronal α3β2 subtype, and CIA and CIB respectively block muscle and neuronal subtypes. GVIIIB caused little or no block on neuronal and muscle nAChRs.

3.5.2 Conotoxin-induced Biological Response

Mice intraperitoneally and fish intramuscularly injected with MII did not show signs of paralysis after conotoxin administration [11], these results are in contrast to α-MI from *C. magus*, which induced paralysis both in mice and fish. In terms of the conotoxins' ability to induce hemolysis, one study reported that neither MII nor Laa-MII caused appreciable hemolysis, even at high concentrations on RBCs isolated from male rats [21]. These findings show that MII is not paralytic and preferentially targets neuronal nAChRs rather than muscle subtypes.

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Intramuscular injection of 3.5 nmol of α-GIC to fish, did not induce paralysis in comparison to α-GI, which caused full paralysis 3 min after administration [13]. Similar observations were also recorded after the intraperitoneal injection of 5 nmol GIC in mice. α-GIC showed no paralytic activity in fish or mice and had no effect on human muscle nicotinic receptor subtypes.

BulA-treated groups significantly expanded the licking time of rats, the Paclitaxel-induced peripheral neuropathy model showed hyperalgesia in BuIA treated group-indicating an analgesic effect. The adverse reactions of BuIA analogs did not affect the autonomic movement of mice, but BuIA weakened the autonomous movement significantly [20]. Moreover, intramuscular injection of CIA to zebrafish caused rapid flaccid paralysis of the skeletal muscle of *Danio rerio* – evidenced by loss of equilibrium of the fish and ultimate immobilization [10]. Paralysis was a dose-dependent effect of CIA with an effective dose of ED_{50} = 110 μg/kg, showing a potent biological effect compatible with a role in prey capture. While 1 mM introduction of CIB, recorded no effect on the locomotion of zebrafish, which is consistent with the absence of activity of CIB on muscle nAChR. These findings suggest the biological role of α-conotoxin for prey capture and the possible therapeutic and drug development potential of piscivorous cone snails derived conotoxins.

3.6 Primary Outcome: Piscivorous mu Conotoxin

3.6.1 Conotoxin Blocked Receptor and Percentage of Blocking

μ-conotoxins GVIIJSSG and GVIIJSH potently inhibit WT rNaV1.2, rNaV1.2[C912A], and rNaV1.2[C918A] with and without the DTT pretreatment [8]. The percentage block for the inhibition of GVIIJSSG without DTT pretreatment on various rNaV1.2), was 73 ± 10% on WT rNaV1.2; 73 ± 11% on rNaV1.2[C912A]; and 20 ± 3% on rNaV1.2[C918A]. While the DTT pretreated GVIIJSSG produced higher inhibition than without DTT pretreatment GVIIJSSG, with recorded inhibition of 89 ± 3% on WT rNaV1.2; 89 ± 3% on rNaV1.2[C912A];

and 90 ± 2% on rNaV1.2[C918A]. The highest percentage block for the inhibition of GVIIJSH was recorded without the DTT pretreatment at 92 ± 4%. Lastly, GVIIJSH with DTT treatment produced percentage block for inhibition of rNaVs 1.2 recorded were 89 ± 3% on WT rNaV1.2; 88 ± 2% on rNaV1.2[C912A]; and 90 ± 2% on rNaV1.2[C918A].

Oocyte-expressing hNaV1.5 recorded a block with an IC_{50} of 0.3 ± 6.5% at 10 μM GVIIJSH and by 10 μM GVIIJSSG with 19 ± 2%. GVIIJSSG readily blocks NaV1.1-1.4, 1.6, and 1.7. In contrast, no block was observed in rNaV1.8 and human NaV1.1 subtypes [3]. GIIIA inhibits the depolarization-evoked NaV1.4 currents in a concentration-dependent manner with an IC_{50} of 0.069 ± 0.005μM [9]. On rat sciatic nerve, GIIIA at 10 μM concentration blocked A-CAPs at 100 ± 0% and C-CAPs at -12.7 ± 15.4% [7]. While BuIIIB at 10 μM blocks A-CAPs at -9.1

± 10.9% and C-CAPs at -7.9 ± 13.3% of rat sciatic nerve. Synthetic variants- μ-BuIIIB and μ-BuIIIC are potent antagonists of NaV1.4 with an average block of ~96%. BuIIIA, on the other hand, blocked NaV1.4 at ~87% [7]. CIIIA and MIIIA at 5 μM on frog DRG

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neurons produced a block of 96 ± 4% and 64 ± 12% after 25-30 minutes of exposure [8].

The conotoxin produced no detectable blocks of the depolarization-activated Na currents at 3 μM [6]. However, TIIIA at 10 μM blocked

A-CAPs at -9.9 ± 13.8% and C-CAPs at -0.2 ± 7.5% of rat sciatic nerve [7].

3.6.2 Conotoxin-Induced Biological Response

Seven analogs of GIIIA and two of GIIIB cause a twitch-tension response [27]. GIIIA caused a dose-dependent inhibition of twitch response with an IC_{50} of approximately 1 μM, with GIIIB causing a more potent inhibition than GIIIA. A study indicated that CIIIA at 0.2 nmol/g: after 4 minutes resulted in allodynia and lethargy, and at 20 minutes, complete paralysis was observed, at a higher concentration of 0.5nmol/g, immediately after injection- lethargy was observed with the animal dying after 13 minutes of exposure owing to respiratory failure; Lastly at 0.55 nmol/g immediate lethargy and hypersensitivity to touch after injection were observed and after 5-8 min death followed [8]. Extracellular recordings of action potentials acquired from three isolated tissue preparations from frogs: skeletal muscle, cardiac muscle, and cutaneous nerves also reported that CIIIA primarily blocks C-CAPs and obliterates action potentials of the three isolated muscles with an IC_{50} value lower than 10 μM [8].

3.7 Primary Outcomes: Molluscivorous Delta Conotoxin

3.7.1 Conotoxin Blocked Receptor and Percentage of Blocking/Inhibition

✿-GmVIA is reported to slow down Na channel inactivation [18], [19]. When K+ and Ca2+ conductance was blocked, the toxin-induced lengthening of the action potential persisted [18]. At final concentrations of 0.3-0.75 μM, a rise in action potential duration by 1-2 orders of magnitude reached over 250 ms in numerous tests. Moreover, 0.75 μM GmVIA did not alter the rise time of the sodium current, but instead, slowed the rate of Na current inactivation- implying that its effect is most likely attributable to a slower rate of Na current inactivation rather than the in Ca2+ and K+ currents [19]. The Na current inactivation kinetics is changed from a single exponential with an average $\tau = 0.47 \pm 0.14$ ms to a slower decay with two-time constants: $\tau = 0.86 \pm 0.12$ms for the initial inactivation phase and $\tau = 488$

± 120 ms for the second phase.

In contrast to the reported activity of GmVIA, ✿-TxVIA produced no detectable effect on Na current peak value and time course while failing to modify Ca influx [22], [23], [24]. It was noted that voltage dependence of the steady-state inactivation of the Na+ current was unaffected, and no current was sustained after treatment [22]. Furthermore, TxVIA could not trigger sodium influx [24], but can reduce the flux enhancement caused by CsTx almost twofold. On high nanomolar concentrations, TxVIA partially inhibited human Cav3.2, produced little effect on Cav3.3, and promoted the opening of Cav3.1 [23]. Moreover, the 60 μM TxVIA only inhibited the Cav3.2 by 42%. Although 0.5 μM TxVIA has decreased the inactivation of molluscan NaV current, 5 μM TxVIA did not influence human NaV responses in SH-SY5Y cells. TxVIA (10M) does not affect calcium

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influx in HEK cells transiently expressing mouse NaV1.7.

3.7.2 Conotoxin-Induced Biological Response

Administration of 20 nmol ✿-GmVIA into local garden snail resulted in the retraction of the head and body into the shell, followed by a release of viscous green slime and convulsive undulation of the snail into and out of the shell. Biological effects on garden snails were detectable at a level of 1.25 nmol/g, and are more evident at 2 nmol/g. No noticeable biological activity was observed from 10 nmol/g injected peritoneally [18].

Meanwhile, TxVIA did not affect the rats, even when given at a level of 30 nmol/rat. When the two toxins are given together, 30nmol TxVIA protects the rats from the toxic effects of 0.5nmol CsTx (more than twice the lethal dose), demonstrating that TxVIA operates as an antagonist in the rat brain. Within the time range of the trial, this protective effect appears to be absolute on the dose used [24]. Moreover, zebrafish injected with 250ng/100mg TxVIA displayed less swimming activity than control fish. However, this effect was insignificant and soon reversed to regular edge swimming. Furthermore, the lack of swimming bursts or abnormal swimming behavior following the TxVIA injection suggests that pain pathways were not triggered [23]. TxVIA at ED50 of 36.0 (pmol/100mg) causes paralysis in snails but does not show any activity in vertebrates. These findings suggest that GmVIA causes a slower rate of sodium current inactivation, while it cannot modify the sodium current peak and inactivation kinetics [22].

3.8 Primary Outcomes: Molluscivorous Omega Conotoxin

3.8.1 Conotoxin Blocked Receptor and Percentage of Blocking/Inhibition

The activity of ω-TxVII was observed on the Ca channel-blocking activity in the neuronal culture of *Lymnaea stagnalis* [25], [26]. Application of 20 μm synthetic TxVII blocked the L-type HVA calcium channel significantly and reversibly from the *L. stagnalis* neurons (RPeD1 cells). A Ca current blockade and depolarizing pulses occurred. Moreover, it reduced peak L-type-like Ca currents by 29±14%. The effect of conotoxin was more pronounced at the end of the depolarization pulse, where the full amplitude was reduced by 72±15%. However, equally applied conotoxin exhibited a low efficacy on the L-type Ca channel in PC23 cells of rat origin, which produced a calcium flux of 111.5±13.2%. Moreover, the result indicates the low efficacy of conotoxin in effectively blocking L-type Ca channels in PC12 cells [26]. On the other hand, TxVII mimics the effects of nimodipine in preferentially blocking the sustained calcium current. Administration of 10 μm TxVII blocked the sustained current of L-type HVA Ca+ current of about 85% at +10mV and by 95% at 40mV. A dose-dependency for TxVII indicated the threshold concentration of 100 nM as the minimal amount and 10 μm for the maximal effect. No significant effect from the same amount of toxin was observed on voltage-dependent Na or K currents [25].

3.8.2 Conotoxin-Induced Biological Response

TxVII was reported to block DHP-sensitive calcium channels of the caudodorsal cell (CDC) neurons of *L. stagnalis*, expressing subtypes of HVA calcium current essential in

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hormone release regulation. The same results were presented that utilized synthesized and purified peptides and recorded the block of L-type calcium currents. The toxin did not affect the voltage-dependent properties of the Ca channel [26]. These effects of TxVII on molluscan neurons would prove helpful for studies on the roles of Ca channels in synaptic mechanisms in this system, more so on which respiratory system is generated in intact animals, as observed in RPeD1. The absence of effect from TxVII, as it did not block L-type Calcium channels in PC12 cells, suggests that the L-type channel may reveal either a subtype or phyletic subdivision, which would be distinguishable by using the conotoxin.

4. DISCUSSION

Marine cone snails contain a highly diverse and unique set of peptides that shows promising potential as pharmacological tools in understanding various ion channels, receptors, and transporter. With only a fraction of conotoxins studied, the need for more studies concerning the potential pharmacological and therapeutic application of conotoxins is required, and rigorous analysis of the evidence presented on the current knowledge regarding the mechanism and pharmacological effects of conotoxins is crucial to produce evidence with confidence and certainty. As such, this review was performed to produce pooled data on the effects of conotoxins on various channels and receptors and the conotoxins' induced biological. Data from twenty-seven (27) studies provided level 3 evidence and a serious risk of bias due to a lack of blind outcome assessment. The heterogeneity of the study, comparator, and outcome measure make it impracticable for a meta-analysis. The results of the review should be interpreted with the following caveats in mind: There were different anatomical areas used for observing the conotoxins' effect on in vivo and in vitro biological assays, and not all papers specify the location. The appropriateness and similarity of comparators have not been consistent throughout the studies included, and the variability in outcome measurement precluded a meta-analysis from being conducted.

4.1 Conotoxin Biological Activity

The results presented should be interpreted with the context that the studies assessed have a serious risk of bias in the domain of bias in the measurement of outcome. The results for the activity of GID have been consistent, of the two included studies both reported the potency and selectivity of the toxin on α7, α3β2, and α4β2 nAChRs, with consistent IC_{50} values observed [15], [17,]. These findings regarding the selectivity and affinity of GID conotoxin are consistent with more recent studies [30], [31] as a similar affinity for neuronal nicotinic receptors was observed. Additionally, GID conotoxin was similarly reported to have a higher affinity for α7 and α4β2 compared to α3β2 receptors [30], [31]. Due to the high affinity to the human α7 subtype, this conotoxin is a valuable probe for understanding the physiological roles of these receptors [31]. αS-GVIIIB caused little or no block on rat neuronal and human muscle nAChR subtypes [12]. However, a recent finding suggests that though αS-GVIIIB causes little to no block on human neuronal and muscle nAChRs, the conotoxin is 100-fold more selective for α9α10 receptors than other subtypes [32]. Similarly, αS-GVIIIB, GIC conotoxin was also

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extracted from *C. geographus* and was also unable to block muscle nAChRs but is highly selective for human neuronal (α3β2) nAChRs and is consistent with its inability to produce any signs of paralysis in fish when injected intramuscularly, in contrast to paralytic α-conotoxin GI [13]. Results also consistently showed that MII causes a dose-dependent block of α3β2 receptors at nanomolar concentrations [11], [14], [21]. Similar to GIC, intraperitoneal and intramuscular injection of MII into rats and fish produced no signs of paralysis, in contrast to MI [11]. Only one study reported that MII has no appreciable hemolytic effect on rat red blood cells [21]. Findings regarding the selectivity of α-conotoxin MII for the α6 subtype are potentially important in nicotine addiction [33].

BuIA distinguishes and targets specific ligand binding sites from different nAChR subunits. Rats treated with BuIA conotoxin have a significantly higher licking time in the hot plate test and higher hyperalgesia in the paclitaxel model [20]; however, BuIA is not compared to any frequently referenced substances for comparison. This study utilized rat samples and adverse effects such as the weakened autonomous movement to measure the biological effects of conotoxin. Oocytes expressing muscle-type nAChR were potently blocked by CIA, while neuronal subtypes were blocked at a lower affinity. In contrast, CIB potently blocks neuronal nAChRs but has no recorded effect on muscle subtypes. Zebrafish treated with CIA experienced a rapid flaccid paralysis of skeletal muscle, while CIB did not produce any noticeable effect on zebrafish locomotion. The evidence suggests that alpha- conotoxins are an excellent tool for determining and discriminating between neuronal and muscle nAChRs. However, scarce and uncertain pieces of evidence on conotoxins' effects on in vivo and in vitro biological assays do not show to have more beneficial analgesic and pharmacological effects, compared to other frequently referenced substances. μ-conotoxins of all piscivorous *Conus* species included in the systematic review have been discussed and are highly specific for sodium channels [3], [4], [5], [6], [7], [8], [9], [27], [28], [29]. GIIIA (10 μM) also potently blocks A-CAPS at 100±0% but incompletely blocks C-CAPs at

-12.7±5.4% in rat sciatic nerves [7].

Other conotoxins of *C. bullatus*, BuIIIB and BuIIIC, were more potent sodium channel blockers for NaV subtypes with a block of 96% compared to BuIIIA at ~87% % [4]. BuIIIB at 10 μM also blocks A-CAPs at -9.1±10.9% and C-CAPs at -7.9±13.3% [7].

CIIIA at 5 μM produced a block of 96±4%, while MIIIA at 64±12% in TTX-resistant sodium channels. The potency of the conotoxins exhibited lethal results as observed in frog DRG neurons than in treated frog skeletal and cardiac muscle [29]. Lethal symptoms were observed in bioassays done in an intracranial injection of CIIIA to mice, which produced severe results after administration of high conotoxin concentration, starting from 0.2 nmol/g resulting in paralysis after 37 minutes, while the highest dose of 0.55 nmol/g induced death 5-8 minutes after the conotoxin introduction. The inhibitory activity of TIIIA was observed in rat NaVs at 3 μM concentration; the toxin blocked rNaV 1.2 by ~95% with an IC_{50} of 0.04 μM [6]. TIIIA did not produce any significant blockade when tested for inhibition on TTX-sensitive VGSC channels in similar concentrations. TIIIA at 10 Mm inhibits A-CAPs at -

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9.9±13.8% and C-CAPs at -0.2±7.5% [7]. Results discussed must be interpreted with the context that the assessors are aware of the group receiving the type of intervention [4], [5], [6], [29]. The comprehensive understanding of μ-conotoxins from piscivorous *Conus* species as sodium channel blockers is significant in improving therapeutic tools. Knowing that the conotoxins of these snails can induce death as quickly as possible serve as a powerful tool for prey capture and shows potential as a pharmacological tool. However, blinded RCTs regarding the therapeutic application and effect of conotoxin are needed to produce evidence with high certainty and confidence.

The results on δ-conotoxin GmVIA consistently reported that the mechanism underlying the effect of GmVIA is to slow down sodium current inactivation rather than altering Ca2+ and K+ currents. Of the two (2) included studies, both reported a reduction in calcium current and the conotoxin-induced prolongation of action potential extending to over 250 ms [18] to 370 ms [19]. Additionally, the effects of the conotoxin of *C. gloriamaris* on limpet snails showed the retraction of head and body into the shell, followed by the animal secreting a viscous green slime and convulsive undulation into and out of the shell after administration of conotoxin into the animal [18]. Three included studies reported similar effects of TxVIA conotoxin on sodium channels. Moreover, there were no detectable effects or prolonged inactivation of sodium currents in humans, frog skeletal fibers, and rat brain synaptosomes [22], [23], [24].

Limpet snails [22] and mice [24] showed no signs of paralysis after the injection of conotoxin. TxVIA inhibited Cav3.2, indicating a possible pain-relieving activity. However, the conotoxin activation of Cav1.3 indicates its possible pain-inducing effect. Whereas zebrafish injected intramuscularly with TxVIA showed neither signs of pain-related behaviors and paralysis nor other adverse effects [23].

At present, the results on the pain-relieving effect of TxVIA are still scarce and further studies are required to provide evidence that is of high quality and certainty. The Ca2+ channel-blocking activity of ω-TxVII requires further analyses to highlight the conotoxins' degree of specificity for L-type or DHP-sensitive calcium channels [25], [26]. Concentrations of 0.1-20 μm induce up to 72±15 - 95% L-type calcium channel blocking in RPeD1 cells. The reduction of L-type HVA calcium currents in the molluscan neuronal culture of *L. stagnalis* could be interpreted as the observed efficacy of the *C. textile* toxin for mollusk prey. Meanwhile, similar experiments done in PC12 cells of rat origin exhibited differing results in the rate of efficacy and percentage of blocking. The contrasting results suggest TxVII and other toxins serve as excellent pharmacological tools in distinguishing subtype and phyletic subdivision of the L-type channel family. The results presented should be interpreted with the context that each study was assessed to have a serious risk of bias [25], [26].

5. CONCLUSION

There is low certainty of evidence regarding selected conotoxins' analgesic and therapeutic activity. This means that the reviewers do not know with certainty whether intraperitoneal, intracranial, and intramuscular injection of conotoxin to animals causes pain relief and would provide similar effects on humans, since indirect surrogate ways

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of measurement were utilized. Moreover, randomized trials that provide certainty about the conotoxins' analgesic effect are scarce. However, results suggest that MII, GIC, and CIB conotoxins are non-paralytic to fish and mice compared to paralytic α-conotoxins MI, GI, and CIA. μ-conotoxin CIIIA can cause lethargy and allodynia at a dose of 0.2 nmol/g and could cause death in mice at 0.55 nmol/g. ✿-conotoxin GmVIA causes convulsive undulation into and out of the shell of snails, while TxVIA does not affect rats. And ω-conotoxin TxVII does not affect molluscan calcium channels. Blinded randomized control trials regarding the therapeutic and application as a pharmacological compound drug of conotoxin may improve confidence in concluding the analysis. Overall, there is strong evidence that conotoxins are a valuable tool for understanding the structure-function of numerous channels and receptors and a great discriminant on voltage- and ligand-gated ion channel subtypes. Studies focusing on these levels are highly recommended for advancing our understanding of the pharmaceutical applications of conotoxins.

References

1. T. Lancet, "A time of crisis for the opioid epidemic in the USA," The Lancet, vol. 398, no. 10297, p. 277, 2011, doi:10.1016/ S0140-6736(21)01653-6. (Editorial)
2. M. Chrisholm-Burns, C. Spivey, E. Sherwin, J. Wheeler, and K. Hohmeier, "The opioid crisis: Origins, trends, policies, and the roles of pharmacists," American
3. Journal of Health-System Pharmacy, vol. 76, no. 7, pp.424-435, 2019, doi:10.1093/ajhp/zxy089. (Journal or magazine citation)
4. J. Gajewiak, L. Azam, J. Imperial, A. Walewska, B. Green, P. Bandyopadhyay, S. Raghuraman, B. Ueberheide, M. Bern, H.M. Zhou, N.A. Minassian, R.H. Hagan,
5. M. Flinspach, Y. Liu, G. Bulaj, A.D. Wickenden, B.M. Olivera, D. Yoshikami, and M. Zhang, "A disulfide tether stabilizes the block of sodium channels by the conotoxin O-GVIIJ," Proceedings Of The National Academy Of Sciences, vol. 111, no. 7, pp. 2758-2763, 2013, doi:10.1073/pnas.1324189111. (Journal or magazine citation)
6. M. Holford, M. Zhang, K.H. Gowd, L. Azam, B.R. Green, M. Watkins, J. Ownby, D. Yoshukami, G. Bulaj, and B.M. Olivera, "Pruning nature: Biodiversity-derived discovery of novel sodium channel blocking conotoxins from *Conus* bullatus," Toxicon, vol. 53, no. 1, pp. 90-98, 2009, doi:10.1016/j.toxicon.2008.10.017. (Journal or magazine citation)
7. Z. Kuang, M. Zhang, K. Gupta, J. Gajewiak, J. Gulyas, P. Balaram, J.E. Rivier, B.M. Olivera, D. Yoshikami, G. Bulaj, and R.S. Norton, "Mammalian Neuronal Sodium Channel Blocker μ-Conotoxin BuIIIB Has a Structured N-Terminus That Influences Potency," ACS Chemical Biology, vol. 8, no. 6, pp. 1344-1351, 2013, doi:10.1021/cb300674x. (Journal or magazine citation)
8. R. Lewis, C. Schroeder, J. Ekberg, K.J. Nielsen, M. Loughnan, L. Thomas, D.A. Adams, R. Drinkwater, D.J. Adams, and P.F. Alewood, "Isolation and Structure- Activity of μ-Conotoxin TIIIA, A Potent Inhibitor of Tetrodotoxin-Sensitive Voltage-Gated Sodium Channels," Molecular Pharmacology, vol. 71, no. 3, pp. 676- 685, 2006, doi:10.1124/mol.106.028225. (Journal or magazine citation)
9. M. Wilson, D. Yoshikami, L. Azam, J. Gajewiak, B.M. Olivera, G. Bulaj, and M. Zhang, "μ-Conotoxins that differentially block sodium channels NaV1.1 through

ISSN: 1671-5497
E-Publication: Online Open Access
Vol: 42 Issue: 06-2023
DOI 10.17605/OSF.IO/CYH9N

10. 1.8 identify those responsible for action potentials in sciatic nerve," Proceedings Of The National Academy Of Sciences, vol. 108, no. 25, pp. 10302-10307, 2011, doi:10.1073/pnas.1107027108. (Journal or magazine citation)
11. M. Zhang, J. Gajewiak, L. Azam, G. Bulaj, B.M. Olivera, and D. Yoshikami, "Probing the Redox States of Sodium Channel Cysteines at the Binding Site of µO§- Conotoxin GVIIJ," Biochemistry, vol. 54, no. 25, pp. 3911-3920, 2015, doi:10.1021/acs.biochem.5b00390. (Journal or magazine citation)
12. P. Han, K. Wang, X. Dai, Y. Cao, S. Liu, H. Jiang, C. Fan, W. Wu and J. Chen, "The Role of Individual Disulfide Bonds of µ -Conotoxin GIIIA in the Inhibition of
13. NaV1.4," Marine Drugs, vol. 14, no. 11, pp. 213, 2016, doi:10.3390/md14110213. (Journal or magazine citation)
14. J. Giribaldi, D. Wilson, A. Nicke, Y. Hamdaoui, G. Laconde, A. Faucherre, H. Maati, N.L. Daly, C. Enjalbal, and S. Dutertre, "Synthesis, Structure and Biological Activity of ClA and ClB, Two α-Conotoxins from the Predation-Evoked Venom of *Conus* catus," Toxins, vol. 10, no. 6, pp. 222, 2018, doi:10.3390/toxins10060222. (Journal or magazine citation)
15. G. Cartier, D. Yoshikami, W. Gray, S. Luo, B.M. Olivera, and J. McIntosh, "A New α-Conotoxin Which Targets α3β2 Nicotinic Acetylcholine Receptors," Journal
16. Of Biological Chemistry, vol. 271, no. 13, pp. 7522-7528, 1996, doi:10.1074/jbc.271.13.7522. (Journal or magazine citation)
17. S. Christensen, P. Bandyopadhyay, B.M. Olivera, and J. McIntosh, "αS-conotoxin GVIIIB potently and selectively blocks α9α10 nicotinic acetylcholine receptors,"
18. Biochemical Pharmacology, vol. 96, no. 4, pp. 349-356, 2015, doi:10.1016/j.bcp.2015.06.007. (Journal or magazine citation)
19. J. McIntosh, C. Dowell, M. Watkins, J. Garrett, D. Yoshikami, and B.M. Olivera, "α-Conotoxin GIC from *Conus* geographus, a Novel Peptide Antagonist of Nicotinic
20. Acetylcholine Receptors," Journal of Biological Chemistry, vol. 277, no. 37, pp. 33610-33615, 2002, doi:10.1074/jbc.m205102200. (Journal or magazine citation)
21. D. Everhart, G. Cartier, A. Malhotra, A. Gomes, J. McIntosh, and C. Luetje, "Determinants of Potency on α-Conotoxin MII, a Peptide Antagonist of Neuronal
22. Nicotinic Receptors," Biochemistry, vol. 43, no. 10, pp. 2732-2737, 2004, doi:10.1021/bi036180h. (Journal or magazine citation) Nicke, M.L. Loughnan, E.L. Millard, P.F. Alewood, D.J. Adams, N.L. Daly, D.J. Craik, and R.J. Lewis, "Isolation, Structure, and Activity of GID, a Novel α4/7- Conotoxin with an Extended N-terminal Sequence," Journal Of Biological Chemistry, vol. 278, no. 5, pp. 3137-3144, 2002, doi:10.1074/jbc.m210280200.
23. H. Kim and J. McIntosh, "α6 nAChR subunit residues that confer α-conotoxin BuIA selectivity," Th e FASEB Journal, vol. 26, no. 10, pp. 4102-4110, 2012, doi:10.1096/fj.12-204487. (Journal or magazine citation)
24. E.L. Millard, S.T. Nevin, M.L. Loughnan, A. Nicke, R.J. Clark, P.F. Alewood, R.J. Lewis, D.J. Adams, D.J. Craik, and N.L. Daly, "Inhibition of Neuronal Nicotinic Acetylcholine Receptor Subtypes by α-Conotoxin GID and Analogues," Journal Of Biological Chemistry, vol. 284, no. 8, pp. 4944 -4951, 2008, doi:10.1074/jbc.m804950200. (Journal or magazine citation)
25. K.J. Shon, A. Hasson, M.E. Spira, L.J. Cruz, W.R. Gray, and B.M. Olivera, "Delta-conotoxin GmVIA, a novel peptide from the venom of *Conus* gloriamaris,"
26. Biochemistry, vol. 33, no. 38, pp. 11420–11425, 1994, doi:10.1021/bi00204a003. (Journal or magazine citation)

ISSN: 1671-5497
E-Publication: Online Open Access
Vol: 42 Issue: 06-2023
DOI 10.17605/OSF.IO/CYH9N

27. Hasson, K. Shon, B.M. Olivera, and M. Spira, "Alterations of voltage-activated sodium current by a novel conotoxin from the venom of *Conus* gloriamaris,"

28. Journal of Neurophysiology, vol. 73, no. 3, pp. 1295-1301, 1995, doi:10.1152/jn.1995.73.3.1295. (Journal or magazine citation)

29. C. Liu, P. Wu, H. Zhu, P. Grieco, R. Yu, X. Gao, G. Wu, D. Wang, H. Xu, and W. Qi, "Rationally Designed α-Conotoxin Analogues Maintained Analgesia Activity and

30. Weakened Side Effects," Molecules, vol. 24, no. 2, pp. 337, 2019, doi:10.3390/molecules24020337. (Journal or magazine citation)

31. J.T. Blanchfield, J.L. Dutton, R.C. Hogg, O.P. Gallagher, D.J. Craik, A. Jones, D.J. Adams, R.J. Lewis, P.F. Alewood, and I. Toth, "Synthesis, Structure Elucidation, in Vitro Biological Activity, Toxicity, and Caco-2 Cell Permeability of Lipophilic Analogues of α-Conotoxin MII," Journal Of Medicinal Chemistry, vol. 46, no. 7, pp. 1266-1272, 2003, doi:10.1021/jm020426j. (Journal or magazine citation)

32. Shichor, M. Fainzilber, M. Pelhate, C. Malecot, E. Zlotkin, and D. Gordon D, "Interactions of b-Conotoxins with Alkaloid Neurotoxins Reveal Differences Between the Silent and Effective Binding Sites on Voltage-Sensitive Sodium Channels," International Society for Neurochemistry, vol. 67, no. 6, pp. 2451–2460, 1996, doi: 10.1046/j.1471-4159.1996.67062451.x. (Journal or magazine citation)

33. D. Wang, S. Himaya, J. Giacomotto, M. Hasan, F. Cardoso, L. Ragnarsson, and R. Lewis, "Characterisation of δ-Conotoxin TxVIA as a Mammalian T-Type Calcium

34. Channel Modulator," Marine Drugs, vol. 18, no. 7, pp. 343, 2020, doi:10.3390/md18070343. (Journal or magazine citation)

35. M. Fainzilber, O. Kofman, E. Zlotkin, and D. Gordon, "A new neurotoxin receptor site on sodium channels is identified by a conotoxin that affects sodium channel inactivation in molluscs and acts as an antagonist in rat brain," Journal of Biological Chemistry, vol. 269, no. 4, pp. 2574 -2580, 1994. (Journal or magazine citation)

36. M. Fainzilber, J.C. Lodder, R. van der Schors, K.W. Li, Z. Yu, A.L. Burlingame, W.P. Geraerts, and K.S. Kits, " A Novel Hydrophobic ω-Conotoxin Blocks Molluscan Dihydropyridine-Sensitive Calcium Channels," Biochemistry, vol. 35, no. 26, pp. 8748–8752, 1996, doi:10.1021/bi9602674. (Journal or magazine citation)

37. T. Sasaki, Z. Feng, R. Scott, N. Grigoriev, N. Syed, M. Fainzilber, and K. Sato, "Synthesis, Bioactivity, and Cloning of the L-Type Calcium Channel Blocker ω- Conotoxin TxVII," Biochemistry, vol. 38, no. 39, pp. 12876-12884, 1999, doi:10.1021/bi990731f. (Journal or magazine citation)

38. K. Sato, Y. Ishida, K. Wakamatsus, R. Kato, H. Honda, Y. Ohizumi, H. Nakamura, M. Ohyag, J. Lamelin, D. Kohda, and F. Inagaki F, "Active Site of p-Conotoxin GIIIA, a Peptide Blocker of Muscle Sodium Channels," The Journal of biological chemistry, vol. 266, no. 26, pp. 16989–16991, 1991. (Journal or magazine citation)

39. K. Sato, Y. Yamaguchi, Y. Ishida, and Y. Ohizumi, "Roles of Basic Amino Acid Residues in the Activity of μ-Conotoxin GIIIA and GIIIB, Peptide Blockers of Muscle

40. Sodium Channels," Chemical Biology & Drug Design, vol. 85, no. 4, pp. 488-493, 2015, doi:10.1111/cbdd.12433. (Journal or magazine citation)

41. M.M. Zhang, B. Fiedler, B.R. Green, P. Catlin, M. Watkins, J.E. Garrett, B.J. Smith, D. Yoshikami, B.M. Olivera, and G. Bulaj, "Structural and Functional Diversities among μ-Conotoxins Targeting TTX-resistant Sodium Channels," Biochemistry, vol. 45, no. 11, pp. 3723-3732, 2006, doi:10.1021/bi052162j. (Journal or magazine citation)