



HAL
open science

Use of Venom Peptides to Probe Ion Channel Structure and Function

Sébastien Dutertre, Richard J Lewis

► **To cite this version:**

Sébastien Dutertre, Richard J Lewis. Use of Venom Peptides to Probe Ion Channel Structure and Function. *Journal of Biological Chemistry*, 2010, 285 (18), pp.13315-13320. 10.1074/jbc.R109.076596 . hal-02306886

HAL Id: hal-02306886

<https://hal.science/hal-02306886v1>

Submitted on 7 Oct 2019

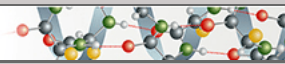
HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Minireviews:

**Use of Venom Peptides to Probe Ion
Channel Structure and Function**

PROTEIN STRUCTURE
AND FOLDING



Sébastien Dutertre and Richard J. Lewis

J. Biol. Chem. 2010, 285:13315-13320.

doi: 10.1074/jbc.R109.076596 originally published online February 26, 2010

Access the most updated version of this article at doi: [10.1074/jbc.R109.076596](https://doi.org/10.1074/jbc.R109.076596)

Find articles, minireviews, Reflections and Classics on similar topics on the [JBC Affinity Sites](#).

Alerts:

- [When this article is cited](#)
- [When a correction for this article is posted](#)

[Click here](#) to choose from all of JBC's e-mail alerts

Supplemental material:

<http://www.jbc.org/content/suppl/2010/03/29/R109.076596.DC1.html>

Read an Author Profile for this article at

<http://www.jbc.org/content/suppl/2010/04/22/285.18.13315.DC1.html>

This article cites 89 references, 33 of which can be accessed free at

<http://www.jbc.org/content/285/18/13315.full.html#ref-list-1>

Use of Venom Peptides to Probe Ion Channel Structure and Function^{*[S]}

Published, JBC Papers in Press, February 26, 2010, DOI 10.1074/jbc.R109.076596

Sébastien Dutertre^{†S1} and Richard J. Lewis^{§2}

From [†]Atheris Laboratories, CH-1233 Bernex-Geneva, Switzerland and the [§]Institute for Molecular Bioscience, University of Queensland, St. Lucia, Queensland 4067, Australia

Venoms of snakes, scorpions, spiders, insects, sea anemones, and cone snails are complex mixtures of mostly peptides and small proteins that have evolved for prey capture and/or defense. These deadly animals have long fascinated scientists and the public. Early studies isolated lethal components in the search for cures and understanding of their mechanisms of action. Ion channels have emerged as targets for many venom peptides, providing researchers highly selective and potent molecular probes that have proved invaluable in unraveling ion channel structure and function. This minireview highlights molecular details of their toxin-receptor interactions and opportunities for development of peptide therapeutics.

Ion channels are a diverse class of membrane proteins that play critical roles in cellular physiology, underlying such essential processes as neuronal signaling and muscle contractility (1). Given these key functions, it is not surprising many toxins have evolved to block or activate ion channels, often with exquisite potency and selectivity. This is most evident among venoms of snakes, spiders, scorpions, insects, cone snails, and sea anemones used for prey capture and predator defense. Their venoms provide a virtually untapped reservoir of millions of bioactive peptides with highly diverse sequences and structures, including many that target ion channels of clinical importance (2). Venom peptides typically act on peripheral targets after injection through a specialized envenomation apparatus such as fangs, stings, harpoons, and nematocysts. Their high potency and target specificity for membrane proteins have been achieved through the evolution of structurally rigid peptide folds with well defined functional faces.

Key ion channel targets of venom peptides include voltage-gated potassium, sodium, and calcium channels and the ligand-gated nicotinic acetylcholine receptors, where toxins acting at different binding sites have evolved across multiple phyla. In addition, a smaller set of venom peptides have evolved to target

the NMDA³ receptor and ASICs. This minireview examines a selection of venom peptides used to probe these ion channels at the functional and structural level.

Potassium Channel Toxins

K⁺ channels are four-domain membrane proteins that selectively transport K⁺ ions across the cell membrane, where they play a key role in regulating cell excitability (3) and non-excitable cell physiology. K⁺ channels comprise two conserved transmembrane segments in each domain, which form the core of the ion-conducting pore and support an extracellular linker that folds back into the channel to form the ion selectivity filter. Apart from these conserved elements, the topology of K⁺ channel subtypes varies greatly, with each domain comprising two, four, or six transmembrane segments. Since the groundbreaking study of the MacKinnon group in 1998 (4), multiple crystal structures of K⁺ channels have expanded our understanding of ion selectivity and gating mechanisms that regulate passage of ions due to changes in membrane potential.

The diversity of K⁺ channels is considerable, with >75 genes identified in mammalian genomes (5), and includes voltage-activated K⁺ channels (K_v), Ca²⁺-activated K⁺ channels (K_{Ca}), inward rectifier K⁺ channels (K_{ir}), and two-pore K⁺ channels (K_{2P}). Thankfully, the venoms of snakes, cone snails, spiders, anemones, and particularly scorpions have provided researchers with potent subtype-selective pharmacological tools, including several with therapeutic potential (2). Many of these peptides possess a conserved functional dyad, comprising a Lys residue near a Tyr, Phe, or Leu, indicative of convergent evolution among many (6) but not all (7) K⁺ channel toxins.

Scorpion—Some 25 years ago, noxiustoxin, isolated from venom of *Centruroides noxius*, was the first identified K⁺ channel-blocking peptide (8). Since then, >120 KTxS ranging from 23 to 64 aa have been sequenced (9). Based on sequence identity and cysteine pairing, scorpion toxins are classified into three subfamilies, the α -, β -, and γ -KTxS. Most KTx structures exhibit a characteristic fold comprising short elements of secondary structure (one α -helix and two or three β -strands). This suggests a conserved binding mode. However, KTxS have evolved to use different faces of their structure to interact with different K⁺ channels (9). For instance, charybdotoxin (KTx1.1) and agitoxin 2 (KTx3.2) interact with the Shaker (or K_v1) channel using residues protruding from the β -strand motif (10, 11), whereas BmPO5 (KTx5.3) binds to the K_{Ca}2.2 channel using residues in the α -helical motif (12).

After the release of the three-dimensional coordinates of the K⁺ channel crystal structure, several groups modeled the interaction of scorpion toxins with their target (12, 13). These models generally agreed with mutagenesis data and helped predict interacting pairs of residues. However, the recent solid-state

* This work was supported in part by a National Health and Medical Research Council program grant and research fellowship (to R. J. L.). This minireview will be reprinted in the 2010 Minireview Compendium, which will be available in January, 2011.

[S] The on-line version of this article (available at <http://www.jbc.org>) contains supplemental Figs. 2 and 3.

¹ Recipient of a long-term postdoctoral fellowship from the European Molecular Biology Organization.

² To whom correspondence should be addressed. E-mail: r.lewis@imb.uq.edu.au.

³ The abbreviations used are: NMDA, N-methyl-D-aspartate; ASIC, acid-sensing ion channel; KTx, K⁺ channel scorpion toxin; aa, amino acid(s); TTX, tetrodotoxin; VGCC, voltage-gated Ca²⁺ channel; nAChR, nicotinic acetylcholine receptor; ECD, extracellular domain; TM, transmembrane segment; AChBP, acetylcholine-binding protein.

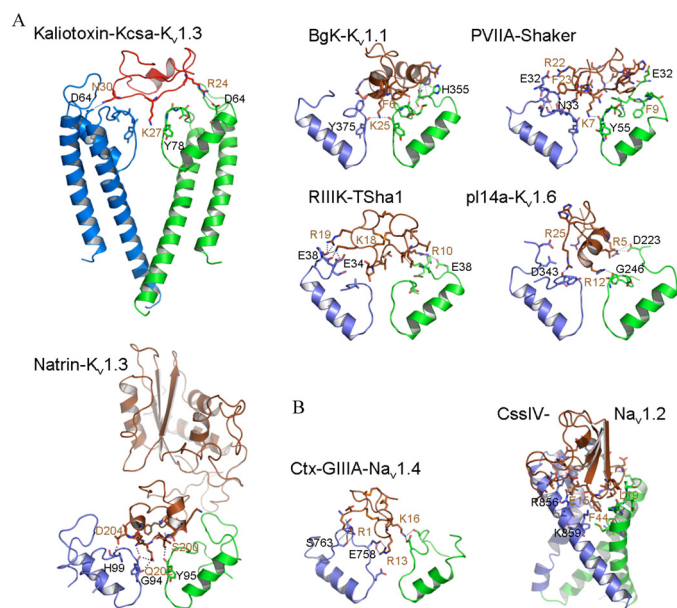


FIGURE 1. Venom peptides in complex with K^+ and Na^+ channels. Interacting residues are shown in stick representation, with toxins in red and the two opposing receptor subunits in blue and green. Except for the kaliotoxin-KcsA-K_v1.3 complex (14), derived from solid-state NMR data, all other toxin-receptor complexes are theoretical models derived from computational simulations. *A*, scorpion, anemone, and cone snail toxin complexes with their respective K^+ channels. For clarity, only the turret selectivity filter of the channel is shown for BgK-K_v1.1 (16), PVIIA-Shaker (21), RIIIK-TSha1 (87), pl14a-K_v1.6 (88), and natrin-K_v1.3 (89) complexes. *B*, cone snail and scorpion toxin complexes with Na^+ channels. μ -Conotoxin GIIIA (Ctx-GIIIA) is shown docked onto the selectivity filter of a Na_v 1.4 model (90), and scorpion toxin CssIV is shown interacting with the voltage sensor of Na_v 1.2 (33).

NMR structure revealed significant rearrangements and/or stabilization of specific conformations in both kaliotoxin and a chimeric K^+ channel that occurred upon toxin binding (14), although the critical Lys²⁷ physically occludes the pore as predicted. This mode of binding appears to be shared with some anemone and cone snail K^+ channel toxins (Fig. 1A).

Sea Anemone—Sea anemones also have yielded a diversity of K^+ channel peptide inhibitors classified into three main types (15). Type 1 toxins comprise 35–37 aa constrained by three disulfide bonds and mostly target K_v1 (Shaker) channels. Structure-activity studies reveal an SKY motif in both ShK (Ser²⁰, Lys²², and Tyr²³) and BgK (Ser²³, Lys²⁵, and Tyr²⁶) toxins that are critical for binding. This SKY motif is absolutely conserved in all type 1 toxins, and docking studies based on double-mutant cycle analysis reveal a plausible mode of interaction (Fig. 1A) (16). Recently, a stable and K_v1.3-selective analog of ShK demonstrated potential in the treatment of autoimmune diseases such as multiple sclerosis and rheumatoid arthritis (17). Type 2 toxins, comprising 58–59 aa and three disulfide bonds, also block K_v1 channels but not as potently as type 1 toxins. Type 2 toxins belong to the Kunitz-type family of peptides and have dual K^+ channel and protease inhibitory activity (18). Type 3 toxins comprise 42–43 aa and three disulfide bonds. BDS-I and BDS-II are selective inhibitors of the K_v3.4 channel, whereas APETx1 is selective for the HERG (human ether-a-go-go-related gene) K^+ channel. Presently, no docking simulations have been reported for these toxins.

Cone Snail—Several toxins isolated from venom of *Conus* species affect K^+ channels. The first discovered was κ -cono-

toxin PVIIA (27 aa) from the venom of *Conus purpurascens* and shown to inhibit the Shaker K^+ channel (19) through the functional dyad comprising Lys⁷ and Phe⁹ plus Lys²⁵ (20). A model of the κ -conotoxin PVIIA-Shaker K^+ channel complex showed a reasonable correlation with experimental data (Fig. 1A) (21). Interestingly, κ -PVIIA can reduce infarct size in rabbit hearts when administered at reperfusion (22). In contrast, the structurally unrelated κ M-RIIK has a distinct binding mode (Fig. 1A) that is apparently not based on a functional dyad (23). A number of conopeptides with unrelated structures have been reported recently, including conkunitzin-S1, which is structurally homologous to snake dendrotoxins, and pl14a from *Conus planorbis*, which was docked onto K_v1.6 (Fig. 1A).

Snake—Isolated >20 years ago from African snakes (*Dendroaspis* sp.), dendrotoxins have proved remarkable tools to study K^+ channel structure and function. These peptides comprise 57–60 aa and three disulfide bonds that stabilize a “Kunitz-type toxin” fold. Initially, the high affinity binding of dendrotoxins was exploited to isolate a K^+ channel protein (24). Later, structure-activity studies of α - and δ -dendrotoxins revealed the importance of the functional dyad, and several models of the interaction of dendrotoxins with K^+ channels have been proposed (25, 26). Other snake toxins also affect K^+ channel activity, including β -bungarotoxin, sarafotoxins, and the much larger natrin. Recently, natrin was docked onto K_v1.3 through a small contact surface (Fig. 1A), suggesting truncated forms of natrin may yield novel channel inhibitors.

Spider—Spider venoms contain “gating modifier peptides” that act at K^+ channels. Hanatoxin, 35-aa three-disulfide bond peptide, binds to the S3b helix of the voltage sensor paddle motif of the K^+ channel (27). Other spider toxins targeting the K^+ channel voltage sensor include guangxitoxin (GxTx1E), which has high affinity for K_v2.1; stromatoxin (ScTx1) and heteroscodratoxins (HmTx1,2), which target K_v2 and K_v4 channels; heteropodatoxins (HpTx1–3), phrixotoxins (PaTx1,2), and TLTx1–3, which preferentially inhibit K_v4 channels; PhTx3-1, which inhibits the outward rectifier A-type K^+ channel; and the Kunitz-type huwentoxin XI, which inhibits trypsin and weakly K_v1.1. Interestingly, the bacterial K_v channel isolated from VsTx1 (28) may allow a spider toxin- K^+ channel co-crystal structure to be solved.

Sodium Channel Toxins

Voltage-sensitive Na^+ channels are four-domain membrane proteins essential for electrical signaling in cells. Nine sodium channel subtypes (Na_v 1–9) have been cloned (1), including some selectively expressed in pain pathways such as Na_v 1.8. A number of Na_v mutations that underlie genetic diseases, including epilepsy and migraines, have been identified.

Seven toxin-binding sites have been described for Na_v channels (sites 1–7) that enhance or inhibit passage of Na^+ ions. The first Na^+ channel inhibitor described was TTX, a poison produced by bacteria and accumulated through the diet of puffer fish. TTX binds in the mouth of the ion-conducting pore at low nM concentrations at TTX-sensitive (Na_v 1.1–1.4, Na_v 1.6, and Na_v 1.7) and μ M concentrations at TTX-resistant (Na_v 1.5, Na_v 1.8, and Na_v 1.9) Na^+ channels. TTX and related saxitoxins

produced by dinoflagellates define site 1 in the pore of the α -subunit of Na^+ channels. Other non-peptidic Na^+ channel poisons include the site 2 activating alkaloid toxins from frogs and plants (batrachotoxin and veratridine), the site 5 activating dinoflagellate polyether toxins (brevetoxins and ciguatoxins) responsible for neurological shellfish poisoning and ciguatera, and the site 7 activating insect-selective plant pyrethroids. In contrast, venom peptides act at sites 1, 3, 4, and 6.

Scorpion—The long chain Na^+ channel toxins found in scorpion venom comprise ~ 64 aa with four disulfide bonds that constrain a highly structured globular $\beta\alpha\beta\beta$ -fold. Two classes of activating scorpion toxins have been characterized. The scorpion α -toxins slow inactivation, and the scorpion β -toxins shift the voltage dependence of activation and first defined sites 3 and 4, respectively (29). Rogers *et al.* (30) provided initial details about receptor site 3 of scorpion α -toxins. Combining the natural variation in peptide sequence with mutational studies is now revealing specific regions important for scorpion β - and α -toxins. For instance, details about the interaction of scorpion β -toxins with receptor site 4 have been uncovered by mutagenesis and double-mutant cycle analysis (31, 32), and a model depicting how a scorpion β -toxin might interact with the voltage sensor of $\text{Na}_v1.2$ has been proposed (Fig. 1B) (33).

Cone Snail—The μ -conotoxins were among the first peptide inhibitors of Na^+ channels identified. These small polar 16–25-aa peptides possess a tightly folded globular structure stabilized by three disulfide bonds. NMR solution structures of SIIIA, TIIIA, PIIIA, GIIIA, GIIIB, KIIIA, and SmIIIA reveal they all have a similar fold except SIIIA, which has a much shorter loop 1 and an helical motif between residues 11 and 16 (34) not seen in the larger μ -conotoxins like TIIIA (35). All μ -conotoxins possess either an exposed Arg or Lys in loop 2 that is important for high affinity interactions with the Na^+ channel. However, its role is less critical for SIIIA and KIIIA, where the pharmacophore has shifted into the helical region of the peptide. μ -Conotoxins preferentially target $\text{Na}_v1.4$ and $\text{Na}_v1.2$ and have weak or no detectable affinity at the validated therapeutic targets $\text{Na}_v1.7$ and $\text{Na}_v1.8$. A docking model depicting how μ -conotoxin GIIIA might plug the selectivity filter of the muscle Na^+ channel ($\text{Na}_v1.4$) is shown in Fig. 1B. This model highlights the critical role of Arg¹³ in the high affinity interaction with $\text{Na}_v1.4$. As our understanding of the architecture of the outer vestibule of the Na^+ channel develops, conotoxins able to block specific Na^+ channel subtypes may be rationally designed.

Cone snail venoms contain two other classes of hydrophobic Na^+ channel toxins, the μO -conotoxins MrVIA and MrVIB isolated from *Conus marmoreus* and the more diverse δ -conotoxins isolated from mollusc- and fish-hunting cone snails. The μO -conotoxins are 31-aa peptides that preferentially block (~ 15 -fold selective) $\text{Na}_v1.8$ and $\text{Na}_v1.4$ over other voltage-gated Na^+ channel subtypes by interfering with the domain II voltage sensor of the Na^+ channel (36). In rats, intrathecal administration of MrVIA was analgesic at doses that produced no local anesthetic-like effects on movement or coordination, revealing that chemical dissection of $\text{Na}_v1.8$ has therapeutic potential (37). MrVIB was also analgesic after peripheral administration, but effects on $\text{Na}_v1.4$ complicate interpretation

of behavioral data. Structure-activity relationships of μO -conotoxins are hampered by difficulties in folding and purifying these peptides efficiently.

The 27–31-residue δ -conotoxins have structures reminiscent of the μO -conotoxins but inhibit Na^+ channel inactivation like the scorpion α -toxins (38). The δ -conotoxins include TxVIA, a selective activator of mollusc Na^+ channels, and EVIA, a selective activator of mammalian neuronal Na^+ channels (39). A cladistics approach is starting to reveal residues that contribute to their structure-activity at Na^+ channels (40). ConoServer provides an online database of conotoxins and conopeptides (41).

Spider—Tx1 from the South American armed spider *Phonetreria nigriventer* was recently found to inhibit μ -conotoxin GIIIB but not TTX binding (42), revealing that pore block can be achieved at multiple overlapping positions in the mouth of Na^+ channels. Perhaps the most interesting spider toxins are gating modifiers that trap Na^+ channels in non-conducting state(s). These include protoxin II from the tarantula *Thrixopelma pruriens*, a 30-aa three-disulfide bond cysteine knot peptide that traps the voltage sensor (43) and preferentially inhibits the pain target $\text{Na}_v1.7$ (44). The slightly larger hainantoxins and huwentoxins from the Chinese bird spider *Ornithoctonus* spp. are also gating modifiers (45). Interestingly, the site of action of huwentoxin IV overlaps site 4 but traps the voltage sensor of domain II in the inward configuration in contrast with scorpion β -toxins, which trap the voltage sensor in an outward configuration (46). Another well studied toxin, Magi 5 from the hexathelid spider *Macrothele gigas*, is less than half the size of the scorpion β -toxins (29 residues) that act at site 4, making it a valuable tool for structure-function relationship studies (47). Magi 5 binds to receptor site 4 on mammalian $\text{Na}_v1.2$ and competes with scorpion β -toxins such as CssIV. A complete alanine scan revealed nine mutants lost biological activity, with an NMR structure attributing these residues to distinct polar and non-polar surfaces (48). Thus, spider toxins provide opportunities for development of subtype-specific Na^+ channel inhibitors.

Not surprisingly given their prey, spiders produce an array of insect-active Na^+ channel toxins, including the +40-aa four-disulfide bond δ -atracotoxins (renamed δ -hexatoxins), which activate Na^+ channels through site 3 by slowing inactivation. Interestingly, recent structural comparisons reveal key residues important for δ -hexatoxin activity appear to be distributed in a similar manner to that seen in the distantly related scorpion α -toxins (49). Given their potent ability to kill insects, spider toxins acting at Na^+ channels also have potential as selective insecticides in agricultural settings (50). ArachnoServer provides a comprehensive online database of spider toxins (51).

Calcium Channel Toxins

VGCCs are structurally related to voltage-gated Na^+ channels. These channels selectively gate the influx of Ca^+ ions required for muscle contraction and neurotransmitter release. In recent years, much has been discovered about the nature of these channels, which have been classified as L-, N-, P-, Q-, T-, and R-types according to their electrophysiological and pharmacological characteristics. Given their diversity and overlapping roles in neurotransmitter release, subtype-selective inhib-

itors are required to determine their relative roles in normal and disease processes. Unfortunately, lack of appropriate crystal structure templates for mammalian calcium channels has limited attempts to generate predictive homology models.

Cone Snail— ω -Conotoxins from fish-hunting cone snails are among the most potent ichthyotoxins. However, it is their selectivity for specific mammalian Ca^+ channel subtypes that has been pivotal in determining the physiological role of specific neuronal Ca^+ channels, especially $\text{Ca}_v2.2$, where ω -conotoxins remain the most selective inhibitors known. The striking ability of subnanomole doses of ω -conotoxin MVIIA or CVID (52) to produce analgesia in rats for up to 24 h when delivered intraspinally (intrathecally) helped identify the pivotal role of N-type VGCCs in spinal pain transmission (53) and led to development of a new class of pain therapeutic, with ω -conotoxin MVIIA (Prialt, Elan Corp.) approved for severe pain. Unfortunately, MVIIA and CVID (AM336 or Leconitide) had unwanted side effects at efficacious doses, possibly a result of on-target effects at inhibitory spinal synapses and supraspinal sites. ω -Conotoxin affinity for N-type Ca^+ channels is reduced upon coexpression with the auxiliary $\alpha_2\delta$ -subunit (54), which might reduce the therapeutic window. Extensive structure-activity studies allow the development of several pharmacophore models for ω -conotoxins, but mimetics with high affinity remain elusive.

Spider—The 48-aa four-disulfide bond ω -agatoxin IVA from grass spider *Agelenopsis aperta* has become a reference inhibitor used to define the role of $\text{Ca}_v2.1$ in cells and tissues (55). The ω -agatoxins are gating modifier toxins that bind to S3 in domain IV, a region that overlaps the binding site of other gating modifier toxins that act on K^+ and Na^+ channels, albeit with different effects on channel gating (56). The 41-residue three-disulfide bond SNX482 (ω -theraphotoxin Hg1a) from the tarantula *Hysteroocrates gigas* interacts with domains III and IV to inhibit activation of VGCCs, including $\text{Ca}_v2.3$ (57). Ca^+ channel modulators from spiders also target insect Ca^{2+} channels and may prove valuable in validating insecticidal Ca^+ channel targets.

Snake—Snake venoms contain a diversity of L-type Ca^+ channel inhibitors. Calciseptine, a 60-aa four-disulfide bond peptide from black mamba *Dendroaspis polylepis polylepis* venom, selectively inhibits dihydropyridine-sensitive cardiac L-type Ca^{2+} channels but enhances L-type current in skeletal muscle (58). Calcicludine from the green mamba *Dendroaspis angusticeps* is a 60-residue three-disulfide bond peptide, resembling the dendrotoxins in structure, that blocks L-type Ca^{2+} currents (59). Calcicludine causes only a partial block of current through interactions across multiple domains to stabilize a low conductance state of the channel in a manner reminiscent of inhibition by dihydropyridines (60).

Nicotinic Acetylcholine Receptors

nAChRs are nonselective pentameric cation channels that open in response to acetylcholine binding (61). The adult muscle nAChR is the major neurotransmitter receptor at the neuromuscular junction and represents a target of choice for many paralyzing toxins. From the 12 neuronal subunits known, few form functional homopentamers (α_7 and α_9), with most native

nAChRs having a subunit stoichiometry that comprises combinations of different α - and β -subunits, e.g. $\alpha_3\beta_2$ or $\alpha_6\alpha_4\beta_2\beta_3$.

The soluble homopentameric AChBP, isolated by the Smit group from *Lymnaea*, is structurally and functionally homologous to the ECD of nAChRs, albeit with low sequence identity (<25%) (61). The structure of AChBP was first solved at atomic resolution by Sixma and co-workers (62), revealing the architecture of the ACh-binding site in great detail. AChBP coordinates have been used to model the ECD of ligand-gated ion channels, especially nAChRs. Alkaloid poisons from plants (nicotine, cytisine, anabaseine, methyllycaconitine, and tubocurarine), poison dart frogs (epibatidine and analogs), algae (anatoxin), and molluscs (neosurugatoxin) act at nAChRs (63), with three toxins (nicotine, epibatidine, and methyllycaconitine) co-crystallized with AChBP (supplemental Fig. 2). Toxins within a structural family appear to bind in a similar manner despite significant differences in primary sequence.

Snake—The snake α -neurotoxins (60–74 aa) are high affinity nAChR ligands first used to help isolate and characterize the nAChR. One of the most studied and widely used is α -bungarotoxin, purified in the 1960s from venom of the banded krait *Bungarus multicinctus* and now co-crystallized with the rat nAChR α_1 -subunit (64). This work represents the first atomic resolution structure of the ECD of a mammalian ligand-gated ion channel component, revealing a remarkable conservation of architecture across species, with the ECD coordinates virtually superimposable with the structure of AChBP bound to the related α -cobratoxin (supplemental Fig. 2). It is evident both α -bungarotoxin and α -cobratoxin utilize a common structural motif comprising a short α -helix to interact with the receptor, confirming the large body of mutagenesis data.

Cone Snail—*Conus* species have evolved a broad range of small α -conotoxins that target nerve and muscle nAChRs for prey capture. A recent screen over the nAChR homolog AChBP revealed the venom of all 30 species examined contained significant nicotinic activity (65), indicating many new ligands remain to be isolated. Indeed, subtype-selective α -conotoxins have significantly contributed to the characterization of nAChRs both *in vivo* and *in vitro*, and some even may have therapeutic applications. Three conotoxins (α -conotoxin ImI and variants of α -conotoxins PnIA and TxIA) have been co-crystallized with AChBP, revealing a similar binding mode within the acetylcholine-binding pocket (65–68). Common among the three structures is the strong contribution of hydrophobic contacts between a conserved proline and several hydrophobic residues of α -conotoxins and residues in the aromatic cage of AChBP (supplemental Fig. 2). Specific electrostatic interactions and hydrogen bonds that are unique for each α -conotoxin establish affinity and selectivity. For instance, the recently discovered α -conotoxin TxIA utilizes a strong electrostatic pairing between Arg⁵ and AChBP Asp¹⁹⁵ binding (65) that contributes to a tilt in its orientation within the acetylcholine-binding pocket. Based on these crystal structures, models for several nAChR subtypes have been built and used as templates to dock α -conotoxins (69–72). Results from mutagenesis experiments using these models were in agreement with the

predictions, highlighting the potential of these docking models to guide ligand design (73).

NMDA Receptors

NMDA receptors are tetrameric ligand-gated ion channels with high Ca^{2+} permeability that mediate fast excitatory neurotransmission in the central nervous system. They are composed mainly of two NR1 and two NR2 subunits and are implicated in chronic and acute neurological disorders. Gouaux and co-workers (74, 75) provided atomic resolution structures of the NMDA extracellular binding domains bound to agonists, partial agonists, and antagonists, opening the way to rational development of NMDA receptor antagonists. To date, only cone snails have yielded venom peptides targeting NMDA receptors.

Conantokins are small peptides (17–22 aa) that selectively and potently inhibit NMDA receptors. The first was isolated from *Conus geographus* (76). Conantokins have potential as analgesics (77) and anticonvulsants (78). Structure-activity studies of conantokins (79) and a docking model suggesting that conantokin G can fit into the agonist-binding cleft of the NR2 subunit (supplemental Fig. 3) (80) should allow rational design of mimetics.

Acid-sensing Ion Channels

ASICs are proton-gated cationic channels in the degenerin/epithelial Na^+ channel superfamily. ASICs play essential roles in the detection and processing of sensory information. In mammals, four genes code for ASICs, but alternative splicing leads to six isoforms (ASIC1a, ASIC1b, ASIC2a, ASIC2b, ASIC3, and ASIC4). Gouaux and co-workers (81) recently determined the x-ray structure of an ASIC1 protein from chicken, which revealed a chalice-like shaped trimer, with each subunit composed of two transmembrane domains, a large multidomain extracellular region, and a negatively charged pocket at the interface of two adjacent subunits forming the proton-binding site. To date, only a sea anemone and a spider venom peptide have been shown to target ASICs, but only the latter has been investigated in detail (82).

PcTx1, a 40-aa peptide from the South American spider *Psalomopoeus cambridgei*, potently and selectively inhibits homomeric ASIC1a ($\text{IC}_{50} < 1 \text{ nM}$) (83). PcTx1 reduces ischemic brain injury in rat and mouse models of ischemia and reduces thermal, mechanical, chemical, inflammatory, and neuropathic pain behaviors in rodents (84). Docking of PcTx1 onto ASIC1 channel (85, 86) suggests it binds at the interface of two subunits, near the proton-binding site, consistent with experimental data.

Conclusions

Venom peptides have evolved to target ion channels using a diversity of structures and modes of interaction. Given that >99% of venom peptides are pharmacologically uncharacterized and that most known toxins target ion channels, venom peptides remain a virtually untapped source of new probes for research and leads to new therapeutics. With the increasing availability of ion channel crystal structure templates, advanced computational tools, and high throughput ion channel assays,

its is becoming viable to rationally design modified venom peptides for improved selectivity, distribution, and *in vivo* plasma half-life, providing an alternative to traditional approaches to ion channel drug discovery.

Acknowledgments—We thank Marc Baldus for preparing a figure of the kaliotoxin-KcsA-K_v1.3 complex and the many colleagues who kindly provided the coordinates of their toxin-receptor complexes.

REFERENCES

- Catterall, W. A. (1995) *Annu. Rev. Biochem.* **64**, 493–531
- Lewis, R. J., and Garcia, M. L. (2003) *Nat. Rev. Drug Discov.* **2**, 790–802
- Yellen, G. (2002) *Nature* **419**, 35–42
- Doyle, D. A., Morais Cabral, J., Pfuetzner, R. A., Kuo, A., Gulbis, J. M., Cohen, S. L., Chait, B. T., and MacKinnon, R. (1998) *Science* **280**, 69–77
- Jenkinson, D. H. (2006) *Br. J. Pharmacol.* **147**, Suppl. 1, S63–S71
- Ménez, A. (1998) *Toxicon* **36**, 1557–1572
- Mouhat, S., De Waard, M., and Sabatier, J. M. (2005) *J. Pept. Sci.* **11**, 65–68
- Carbone, E., Wanke, E., Prestipino, G., Possani, L. D., and Maelicke, A. (1982) *Nature* **296**, 90–91
- Rodríguez de la Vega, R. C., Merino, E., Becerril, B., and Possani, L. D. (2003) *Trends Pharmacol. Sci.* **24**, 222–227
- Ellis, K. C., Tenenholz, T. C., Jerng, H., Hayhurst, M., Dudlak, C. S., Gilly, W. F., Blaustein, M. P., and Weber, D. J. (2001) *Biochemistry* **40**, 5942–5953
- Eriksson, M. A., and Roux, B. (2002) *Biophys. J.* **83**, 2595–2609
- Cui, M., Shen, J., Briggs, J. M., Fu, W., Wu, J., Zhang, Y., Luo, X., Chi, Z., Ji, R., Jiang, H., and Chen, K. (2002) *J. Mol. Biol.* **318**, 417–428
- Rauer, H., Lanigan, M. D., Pennington, M. W., Aiyar, J., Ghanshani, S., Cahalan, M. D., Norton, R. S., and Chandy, K. G. (2000) *J. Biol. Chem.* **275**, 1201–1208
- Lange, A., Giller, K., Hornig, S., Martin-Eauclair, M. F., Pongs, O., Becker, S., and Baldus, M. (2006) *Nature* **440**, 959–962
- Honma, T., and Shiomi, K. (2006) *Mar. Biotechnol.* **8**, 1–10
- Gilquin, B., Braud, S., Eriksson, M. A., Roux, B., Bailey, T. D., Priest, B. T., Garcia, M. L., Ménez, A., and Gasparini, S. (2005) *J. Biol. Chem.* **280**, 27093–27102
- Pennington, M. W., Beeton, C., Galea, C. A., Smith, B. J., Chi, V., Monaghan, K. P., Garcia, A., Rangaraju, S., Giuffrida, A., Plank, D., Crossley, G., Nugent, D., Khaytin, I., Lefevre, Y., Peshenko, I., Dixon, C., Chauhan, S., Orzel, A., Inoue, T., Hu, X., Moore, R. V., Norton, R. S., and Chandy, K. G. (2009) *Mol. Pharmacol.* **75**, 762–773
- Antuch, W., Berndt, K. D., Chávez, M. A., Delfin, J., and Wüthrich, K. (1993) *Eur. J. Biochem.* **212**, 675–684
- Terlau, H., Shon, K. J., Grille, M., Stocker, M., Stühmer, W., and Olivera, B. M. (1996) *Nature* **381**, 148–151
- Jacobsen, R. B., Koch, E. D., Lange-Malecki, B., Stocker, M., Verhey, J., Van Wagoner, R. M., Vyazovkina, A., Olivera, B. M., and Terlau, H. (2000) *J. Biol. Chem.* **275**, 24639–24644
- Huang, X., Dong, F., and Zhou, H. X. (2005) *J. Am. Chem. Soc.* **127**, 6836–6849
- Zhang, S. J., Yang, X. M., Liu, G. S., Cohen, M. V., Pemberton, K., and Downey, J. M. (2003) *J. Cardiovasc. Pharmacol.* **42**, 764–771
- Al-Sabi, A., Lennartz, D., Ferber, M., Gulyas, J., Rivier, J. E., Olivera, B. M., Carlomagno, T., and Terlau, H. (2004) *Biochemistry* **43**, 8625–8635
- Rehm, H., and Lazdunski, M. (1988) *Proc. Natl. Acad. Sci. U.S.A.* **85**, 4919–4923
- Imredy, J. P., and MacKinnon, R. (2000) *J. Mol. Biol.* **296**, 1283–1294
- Katoh, E., Nishio, H., Inui, T., Nishiuchi, Y., Kimura, T., Sakakibara, S., and Yamazaki, T. (2000) *Biopolymers* **54**, 44–57
- Wang, J. M., Roh, S. H., Kim, S., Lee, C. W., Kim, J. I., and Swartz, K. J. (2004) *J. Gen. Physiol.* **123**, 455–467
- Ruta, V., Jiang, Y., Lee, A., Chen, J., and MacKinnon, R. (2003) *Nature* **422**, 180–185
- Rodríguez de la Vega, R. C., and Possani, L. D. (2005) *Toxicon* **46**, 831–844

30. Rogers, J. C., Qu, Y., Tanada, T. N., Scheuer, T., and Catterall, W. A. (1996) *J. Biol. Chem.* **271**, 15950–15962
31. Cohen, L., Ilan, N., Gur, M., Stühmer, W., Gordon, D., and Gurevitz, M. (2007) *J. Biol. Chem.* **282**, 29424–29430
32. Leipold, E., Hansel, A., Borges, A., and Heinemann, S. H. (2006) *Mol. Pharmacol.* **70**, 340–347
33. Cestèle, S., Yarov-Yarovoy, V., Qu, Y., Sampieri, F., Scheuer, T., and Catterall, W. A. (2006) *J. Biol. Chem.* **281**, 21332–21344
34. Schroeder, C. I., Ekberg, J., Nielsen, K. J., Adams, D., Loughnan, M. L., Thomas, L., Adams, D. J., Alewood, P. F., and Lewis, R. J. (2008) *J. Biol. Chem.* **283**, 21621–21628
35. Lewis, R. J., Schroeder, C. I., Ekberg, J., Nielsen, K. J., Loughnan, M., Thomas, L., Adams, D. A., Drinkwater, R., Adams, D. J., and Alewood, P. F. (2007) *Mol. Pharmacol.* **71**, 676–685
36. Leipold, E., DeBie, H., Zorn, S., Borges, A., Olivera, B. M., Terlau, H., and Heinemann, S. H. (2007) *Channels* **1**, 253–262
37. Ekberg, J., Jayamanne, A., Vaughan, C. W., Aslan, S., Thomas, L., Mould, J., Drinkwater, R., Baker, M. D., Abrahamsen, B., Wood, J. N., Adams, D. J., Christie, M. J., and Lewis, R. J. (2006) *Proc. Natl. Acad. Sci. U.S.A.* **103**, 17030–17035
38. Leipold, E., Hansel, A., Olivera, B. M., Terlau, H., and Heinemann, S. H. (2005) *FEBS Lett.* **579**, 3881–3884
39. Barbier, J., Lamthanh, H., Le Gall, F., Favreau, P., Benoit, E., Chen, H., Gilles, N., Ilan, N., Heinemann, S. H., Gordon, D., Ménez, A., and Molgó, J. (2004) *J. Biol. Chem.* **279**, 4680–4685
40. Bulaj, G., DeLaCruz, R., Azimi-Zonooz, A., West, P., Watkins, M., Yoshikami, D., and Olivera, B. M. (2001) *Biochemistry* **40**, 13201–13208
41. Kaas, Q., Westermann, J. C., Halai, R., Wang, C. K., and Craik, D. J. (2008) *Bioinformatics* **24**, 445–446
42. Martin-Moutot, N., Mansuelle, P., Alcaraz, G., Dos Santos, R. G., Cord-eiro, M. N., De Lima, M. E., Seagar, M., and Van Renterghem, C. (2006) *Mol. Pharmacol.* **69**, 1931–1937
43. Sokolov, S., Kraus, R. L., Scheuer, T., and Catterall, W. A. (2008) *Mol. Pharmacol.* **73**, 1020–1028
44. Schmalhofer, W. A., Calhoun, J., Burrows, R., Bailey, T., Kohler, M. G., Weinglass, A. B., Kaczorowski, G. J., Garcia, M. L., Koltzenburg, M., and Priest, B. T. (2008) *Mol. Pharmacol.* **74**, 1476–1484
45. Li, D., Xiao, Y., Hu, W., Xie, J., Bosmans, F., Tytgat, J., and Liang, S. (2003) *FEBS Lett.* **555**, 616–622
46. Xiao, Y., Bingham, J. P., Zhu, W., Moczydlowski, E., Liang, S., and Cummins, T. R. (2008) *J. Biol. Chem.* **283**, 27300–27313
47. Corzo, G., Gilles, N., Satake, H., Villegas, E., Dai, L., Nakajima, T., and Haupt, J. (2003) *FEBS Lett.* **547**, 43–50
48. Corzo, G., Sabo, J. K., Bosmans, F., Billen, B., Villegas, E., Tytgat, J., and Norton, R. S. (2007) *J. Biol. Chem.* **282**, 4643–4652
49. Yamaji, N., Little, M. J., Nishio, H., Billen, B., Villegas, E., Nishiuchi, Y., Tytgat, J., Nicholson, G. M., and Corzo, G. (2009) *J. Biol. Chem.* **284**, 24568–24582
50. King, G. F., Escoubas, P., and Nicholson, G. M. (2008) *Channels* **2**, 100–116
51. Wood, D. L., Miljenović, T., Cai, S., Raven, R. J., Kaas, Q., Escoubas, P., Herzig, V., Wilson, D., and King, G. F. (2009) *BMC Genomics* **10**, 375
52. Lewis, R. J., Nielsen, K. J., Craik, D. J., Loughnan, M. L., Adams, D. A., Sharpe, I. A., Luchian, T., Adams, D. J., Bond, T., Thomas, L., Jones, A., Matheson, J. L., Drinkwater, R., Andrews, P. R., and Alewood, P. F. (2000) *J. Biol. Chem.* **275**, 35335–35344
53. Malmberg, A. B., and Yaksh, T. L. (1995) *Pain* **60**, 83–90
54. Mould, J., Yasuda, T., Schroeder, C. I., Beedle, A. M., Doering, C. J., Zamponi, G. W., Adams, D. J., and Lewis, R. J. (2004) *J. Biol. Chem.* **279**, 34705–34714
55. Mintz, I. M., Venema, V. J., Swiderek, K. M., Lee, T. D., Bean, B. P., and Adams, M. E. (1992) *Nature* **355**, 827–829
56. Winterfield, J. R., and Swartz, K. J. (2000) *J. Gen. Physiol.* **116**, 637–644
57. Bourinet, E., Stotz, S. C., Spaetgens, R. L., Dayanithi, G., Lemos, J., Nargeot, J., and Zamponi, G. W. (2001) *Biophys. J.* **81**, 79–88
58. García, M. C., Hernández-Gallegos, Z., Escamilla, J., and Sánchez, J. A. (2001) *J. Membr. Biol.* **184**, 121–129
59. Schweitz, H., Heurteaux, C., Bois, P., Moinier, D., Romey, G., and Lazdun-ski, M. (1994) *Proc. Natl. Acad. Sci. U.S.A.* **91**, 878–882
60. Wang, X., Du, L., and Peterson, B. Z. (2007) *Biochemistry* **46**, 7590–7598
61. Smit, A. B., Syed, N. I., Schaap, D., van Minnen, J., Klumperman, J., Kits, K. S., Lodder, H., van der Schors, R. C., van Elk, R., Sorgedraeger, B., Brejc, K., Sixma, T. K., and Geraerts, W. P. (2001) *Nature* **411**, 261–268
62. Brejc, K., van Dijk, W. J., Klaassen, R. V., Schuurmans, M., van Der Oost, J., Smit, A. B., and Sixma, T. K. (2001) *Nature* **411**, 269–276
63. Daly, J. W. (2005) *Cell. Mol. Neurobiol.* **25**, 513–552
64. Dellisanti, C. D., Yao, Y., Stroud, J. C., Wang, Z. Z., and Chen, L. (2007) *Nat. Neurosci.* **10**, 953–962
65. Dutertre, S., Ulens, C., Büttner, R., Fish, A., van Elk, R., Kendel, Y., Hop-ping, G., Alewood, P. F., Schroeder, C., Nicke, A., Smit, A. B., Sixma, T. K., and Lewis, R. J. (2007) *EMBO J.* **26**, 3858–3867
66. Ulens, C., Hogg, R. C., Celie, P. H., Bertrand, D., Tsetlin, V., Smit, A. B., and Sixma, T. K. (2006) *Proc. Natl. Acad. Sci. U.S.A.* **103**, 3615–3620
67. Celie, P. H., Kasheverov, I. E., Mordvintsev, D. Y., Hogg, R. C., van Nierop, P., van Elk, R., van Rossum-Fikkert, S. E., Zhmak, M. N., Bertrand, D., Tsetlin, V., Sixma, T. K., and Smit, A. B. (2005) *Nat. Struct. Mol. Biol.* **12**, 582–588
68. Hansen, S. B., Sulzenbacher, G., Huxford, T., Marchot, P., Taylor, P., and Bourne, Y. (2005) *EMBO J.* **24**, 3635–3646
69. Clark, R. J., Fischer, H., Nevin, S. T., Adams, D. J., and Craik, D. J. (2006) *J. Biol. Chem.* **281**, 23254–23263
70. Dutertre, S., Nicke, A., Tyndall, J. D., and Lewis, R. J. (2004) *J. Mol. Recognit.* **17**, 339–347
71. Ellison, M., Gao, F., Wang, H. L., Sine, S. M., McIntosh, J. M., and Olivera, B. M. (2004) *Biochemistry* **43**, 16019–16026
72. Talley, T. T., Olivera, B. M., Han, K. H., Christensen, S. B., Dowell, C., Tsigelny, I., Ho, K. Y., Taylor, P., and McIntosh, J. M. (2006) *J. Biol. Chem.* **281**, 24678–24686
73. Dutertre, S., Nicke, A., and Lewis, R. J. (2005) *J. Biol. Chem.* **280**, 30460–30468
74. Furukawa, H., and Gouaux, E. (2003) *EMBO J.* **22**, 2873–2885
75. Furukawa, H., Singh, S. K., Mancusso, R., and Gouaux, E. (2005) *Nature* **438**, 185–192
76. Mena, E. E., Gullak, M. F., Pagnozzi, M. J., Richter, K. E., Rivier, J., Cruz, L. J., and Olivera, B. M. (1990) *Neurosci. Lett.* **118**, 241–244
77. Malmberg, A. B., Gilbert, H., McCabe, R. T., and Basbaum, A. I. (2003) *Pain* **101**, 109–116
78. Jimenez, E. C., Donevan, S., Walker, C., Zhou, L. M., Nielsen, J., Cruz, L. J., Armstrong, H., White, H. S., and Olivera, B. M. (2002) *Epilepsy Res.* **51**, 73–80
79. Nielsen, K. J., Skjaerbaek, N., Dooley, M., Adams, D. A., Mortensen, M., Dodd, P. R., Craik, D. J., Alewood, P. F., and Lewis, R. J. (1999) *J. Med. Chem.* **42**, 415–426
80. Wittekindt, B., Malany, S., Schemm, R., Otvos, L., Maccacchini, M. L., Laube, B., and Betz, H. (2001) *Neuropharmacology* **41**, 753–761
81. Jasti, J., Furukawa, H., Gonzales, E. B., and Gouaux, E. (2007) *Nature* **449**, 316–323
82. Diochot, S., Salinas, M., Baron, A., Escoubas, P., and Lazdunski, M. (2007) *Toxicon* **49**, 271–284
83. Escoubas, P., De Weille, J. R., Lecoq, A., Diochot, S., Waldmann, R., Champigny, G., Moinier, D., Ménez, A., and Lazdunski, M. (2000) *J. Biol. Chem.* **275**, 25116–25121
84. Mazzuca, M., Heurteaux, C., Alloui, A., Diochot, S., Baron, A., Voilley, N., Blondeau, N., Escoubas, P., Gélot, A., Cupo, A., Zimmer, A., Zimmer, A. M., Eschalié, A., and Lazdunski, M. (2007) *Nat. Neurosci.* **10**, 943–945
85. Pietra, F. (2009) *J. Chem. Inf. Model.* **49**, 972–977
86. Qadri, Y. J., Berdiev, B. K., Song, Y., Lippton, H. L., Fuller, C. M., and Benos, D. J. (2009) *J. Biol. Chem.* **284**, 17625–17633
87. Verdier, L., Al-Sabi, A., Rivier, J. E., Olivera, B. M., Terlau, H., and Carlo-magno, T. (2005) *J. Biol. Chem.* **280**, 21246–21255
88. Mondal, S., Babu, R. M., Bhavna, R., and Ramakumar, S. (2007) *In Silico Biol.* **7**, 0018
89. Wang, F., Li, H., Liu, M. N., Song, H., Han, H. M., Wang, Q. L., Yin, C. C., Zhou, Y. C., Qi, Z., Shu, Y. Y., Lin, Z. J., and Jiang, T. (2006) *Biochem. Biophys. Res. Commun.* **351**, 443–448
90. Tikhonov, D. B., and Zhorov, B. S. (2005) *Biophys. J.* **88**, 184–197