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Essay

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Conus Venom Peptides, Receptor and Ion Channel Targets, and Drug Design: 50 Million Years of Neuropharmacology

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The predatory cone snails (*Conus*) are among the most successful living marine animals (~500 living species). Each *Conus* species is a specialist in neuropharmacology, and uses venom to capture prey, to escape from and defend against predators and possibly to deter competitors. An individual cone snail's venom contains a diverse mixture of pharmacological agents, mostly small, structurally constrained peptides (conotoxins). Individual peptides are selectively targeted to a specific isoform of receptor or ion channel. A variety of such targets have been identified, including many voltage-gated and ligand-gated ion channel subtypes, as well as G protein-linked receptors. Although there are only a few widely shared structural motifs in conotoxins (the majority of the >25,000 peptides in these venoms probably belong to only half a dozen gene superfamilies), the sequences of peptides are remarkably divergent from one *Conus* species to another. We suggest that cone snails undergoing speciation have, in effect, a mutator phenotype which acts specifically on the gene segment encoding the mature toxin region. In their 50 million years of evolution, cone snails anticipated many features of the modern drug industry: disposable hypodermic needles, combination drug therapy, and combinatorial strategies for drug discovery. Recent results indicate that the *Conus* peptide system may provide a novel paradigm for designing ligands that discriminate between closely related members of large families of receptors and ion channels. Many *Conus* peptides may be "Janus-ligands," with two distinct recognition faces oriented in different directions, a design which should make far greater target specificity possible.

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BIOLOGY OF CONE SNAILS: WHY A NEUROPHARMACOLOGICAL STRATEGY?

The carnivorous cone snails (Fig. 1) are relatively young in evolutionary terms; the first fossil *Conus* appear only after dinosaurs went extinct (Kohn, 1990). However, they comprise what is arguably the largest single genus of marine animals living today. All cone snails are venomous predators. It is quite likely that most *Conus* use their venoms for multiple purposes, including prey capture and defense. All 500 living species of cone snails (Kohn, 1976; Röckel *et al*., 1995) have a highly sophisticated venom production apparatus and delivery system (Fig. 2). A hallmark of the latter is the specialized teeth, which in effect serve both as harpoon and disposable hypodermic needle for venom delivery (Kohn *et al*., 1960; Kohn *et al*., 1972).

Although most *Conus* move considerably faster than would be expected of the proverbial snail, they remain relatively slow compared to other ambulatory predators. Cone snails cannot swim; despite this, a significant number have evolved to feed primarily (if not exclusively) on fish (Kohn, 1956). For a predator with these locomotory disadvantages to specialize in such agile prey requires an unusual venom: once the fish-hunting snail strikes its prey, extremely rapid and effective immobilization usually occurs. Given this background biology, it becomes easy to rationalize why the major venom components characterized so far have been found to target key cell surface-signaling components of nervous systems, i.e., ion channels and receptors (Olivera *et al*., 1985, 1990). Slow-moving or sessile venomous predators would have particular need for extremely fast-acting venoms, a scenario favoring evolution of toxins which target nervous systems.

Several features of cone snail venoms have been firmly established in recent years. First, the venoms are extremely complex—venom from an individual *Conus* may have 50–200 distinct, biologically active components. Most of these are small peptides (6–40 amino acids in length), with the majority being in the size range of 12–30 amino acids (see Olivera *et al*., 1990). Although *Conus* toxins are unusually small,

Figure 1. Ten different cone snails. Shown are the shells of ten *Conus* species including, top row: the cloth-of-gold cone, *Conus textile*; the cone of the magi, *Conus magus*. Second row, left to right: the circumcision cone, *Conus circumcisus*; the geography cone, *Conus geographus*; Dusavel's cone, *Conus dusaveli*. Third row, left to right: the glory-of-the-sea cone, *Conus gloriamaris*, the glory-of-India cone, *Conus milneedwardsi*. Bottom row, left to right: the admiral cone, *Conus ammiralis*; the banded marble cone, *Conus bandanus vidua*; Hirasei's cone, *Conus hirasei*. The geography cone, *C. geographus* is responsible for the majority of human fatalities. This species, along with *C. magus*, *C. circumcisus*, and *C. dusaveli*, are all likely to be fish hunting. The other *Conus* shown all hunt other gastropod molluscs, except for *C. hirasei*, which is probably a vermivorous species, although its biology has not been studied.

they are invariably highly constrained conformationally. Most are extensively cross-linked through multiple intramolecular disulfide linkages, although other conformation-constraining strategies, such as the presence of an unusual α-helix-stabilizing posttranslational modification (McIntosh *et al*., 1984; Myers *et al*., 1990; Olivera *et al*., 1990) have been discovered.

The complement of peptides found in any one *Conus* venom is strikingly different from that found in the venom of any other *Conus* species. Thus, in the whole genus, many tens of thousands of distinct pharmacologically active peptides have been evolved. A question which immediately arises is why individual cone snails should need so many different peptides. We speculate that the complement of peptides in a venom may be used for at least three general purposes. An individual peptide may play a role in 1) prey capture, directly or indirectly; 2) defense and escape from predators; or 3) other biological phenomena (such as interaction with potential competitors, for example).

CONE SNAILS CAPTURE PREY USING A COMBINATION DRUG STRATEGY

Although each of the 500 different cone snail species is usually highly specialized with respect to prey, the

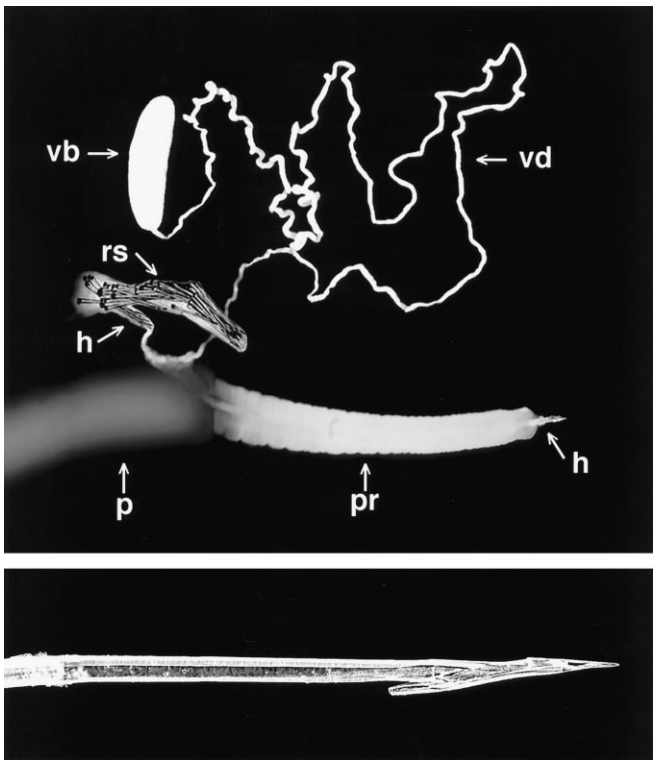

Figure 2. The venom apparatus and harpoon-like tooth of *Conus purpurascens*. Upper panel, a representation of the venom apparatus of *C. purpurascens*. The venom apparatus in all cone snails comprises: vb, a venom bulb which pushes the venom out; vd, the venom duct where the venom is actually made and stored; rs, a radula sac where the harpoon-like teeth are stored; h, harpoon-like teeth; p, pharynx; pr, proboscis, which is used to deliver the harpoon and venom to the prey. The radula sac has been shown in cross-section, to make the harpoons visible. Each harpoon is used only once; in the radula sac they are found in various stages of assembly. Lower panel, a close-up electron micrograph of a single harpoon-like tooth. Venom is ejected through the tooth.

genus as a whole envenomates a surprisingly broad range of prey types. The largest group of *Conus* (>150 species) probably hunt various polychaete worms. However, a substantial number (ca. 70) prey on fish and another ~70 specialize on molluscs. In addition, a number of *Conus* species feed on hemichordates and echiuroids. By far the best understood in terms of molecular mechanisms are the fish-hunting cone snails.

Fish-hunting cone snails can generally be divided into two broad classes: "hook-and-line" fishing snails, which use their long probosces to harpoon prey with a disposable harpoon-like tooth (which also serves as a hypodermic needle) and "net-fishing" cone snails, which engulf prey with a large distensible mouth before stinging (Fig. 3). Among the latter is the geography cone, *Conus geographus*, the species most lethal to man—70% of untreated human stinging cases are fatal (Cruz and White, 1995). Although a hook-and-line strategy would permit prey capture at a greater distance, the potential advantage of net fishing is that it becomes possible to bag a whole school of smaller fish at one time.

For *Conus purpurascens*, which uses a hook-and-line strategy to capture fish, the injected venom has been shown to sequentially elicit two distinct immobilization phases, excitotoxic shock followed by neuromuscular block (Terlau *et al*., 1996). In effect, one group of peptides hyperexcites targeted electrically excitable cells around the venom injection site, whereas a second group of peptide toxins suppresses the motor circuitry of the prey. The first physiological effect, excitotoxic shock, requires the combined action of a peptide which prevents voltage-gated sodium channels from closing once they have opened, thereby increasing the influx of sodium (Shon *et al*., 1995) and a second toxin which blocks certain subtypes of potassium channels, thereby inhibiting potassium efflux (Terlau *et al*., 1996). The combination of increased sodium influx and decreased potassium efflux results in the massive depolarization of neuronal circuitry at the injection site. This has the effect of stunning the fish almost instantaneously upon venom injection.

A second, nonoverlapping, group of toxins in the same venom consists of peptides that act synergistically to block neuromuscular transmission. All fish-hunting *Conus* have a subset of such toxins, which typically include 1) a group of peptides which antagonize different subtypes of presynaptic voltage-gated calcium channels (Olivera *et al*., 1994) and thereby inhibit neurotransmitter release; 2) competitive and noncompetitive antagonists of the postsynaptic nicotinic acetylcholine receptor (Gray *et al*., 1984; Shon *et al*., 1997a) that prevent depolarization at the muscle end plate; and 3) skeletal muscle voltage-gated sodium channel blockers which act like tetrodotoxin (Spence *et al*., 1977; Stone and Gray, 1982; Nakamura *et al*., 1983; Sato *et al*., 1983; Cruz *et al*., 1985); these directly abolish muscle action potentials. Most piscivorous *Conus* have three or four different major peptides which, by acting together, very efficiently wipe out the motor circuitry of the fish prey. The concerted action of this second group is shown in Fig. 4.

The first group of toxins act at the venom injection site to stun prey. Thus, the fish is immobilized during the lag time required for the second group of toxins to reach their targets in the neuromuscular system. By having both groups of toxins, the snail maximizes the probability that the fish will be continuously immobilized after it has been stung, thereby increasing the likelihood that prey will be captured. Both general physiological strategies (stunning the fish by excitotoxic shock, paralysis by neuromuscular block) require the synergistic action of multiple peptides. We

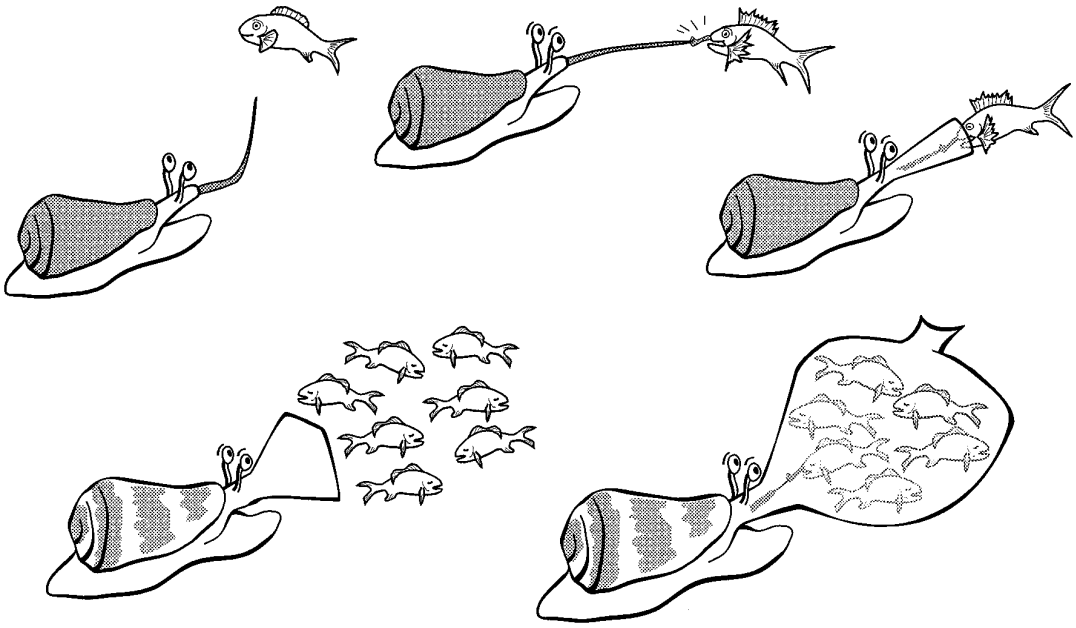

Figure 3. A cartoon representing the hook-and-line (top panel) and the net strategy (bottom panel) of fish-hunting cone snails. *Conus striatus*, *magus*, and *purpurascens* are examples of hook-and-line piscivores. Species such as *Conus tulipa* and *Conus geographus* use a net strategy.

refer to such functionally linked groups of peptides acting together as "toxin cabals." The toxins which cause excitotoxic shock together comprise the "lightning-strike cabal" while those causing neuromuscular block are designated the "motor cabal."

A modern development in pharmacology which has attracted considerable attention is the use of combination drug therapy, particularly for more intractable health problems such as AIDS or incurable tumors. The cone snails appear to have anticipated the development of pharmacological combination strategies by over 40 million years. The peptides which contribute to excitotoxic shock (the lightning-strike cabal) as well as the peptides that disrupt neuromuscular transmission (the motor cabal) comprise, in effect, a highly sophisticated application of a combination drug strategy in a natural system. (Since the relevant "drugs" are hardly beneficial to the prey, it seems inappropriate to refer to this as combination drug "therapy").

In addition to the two toxin cabals directly involved in prey immobilization, other venom peptides may play accessory roles to enhance the probability of prey capture. For example, there is evidence for peptides that suppress the fight-or-flight response of the fish prey (Cartier *et al*., 1996; Tavazoie *et al*., 1997). In addition, vasopressin-like peptides which constrict arteries may promote more rapid uptake of paralytic peptides of the motor cabal by the capillary bed (Cruz *et al*., 1987). The impression created by the pharmacological characterization carried out so far is of an extremely strong selection pressure for very rapid prey immobilization and a biological system able to mount a sophisticated response in evolutionary time to address this pressure.

GENERATING NEUROPHARMACOLOGICAL DIVERSITY THROUGH A COMBINATORIAL LIBRARY SEARCH STRATEGY

As more *Conus* peptides have been analyzed, it has become apparent that the venoms from different species have peptides surprisingly divergent in sequence from each other. Very rapid evolution of novel venom components has apparently occurred during the radiation of these molluscs in the last ~50 million years. As a result, homologous peptides from different species often have diverged to the point of making any sequence similarity unrecognizable. This is illustrated by the structurally diverse peptides shown in Fig. 5, all of which inhibit the postsynaptic nicotinic acetylcholine receptor at the neuromuscular junction.

Although the molecular mechanisms that lead to rapid interspecific divergence are not understood, the phenomenon has become better defined. *Conus* peptides are initially translated as larger prepropeptide precursors; a mature *Conus* peptide of 20 amino acids is generally processed from a 70 to 80 amino acid precursor, with a single nonrepeated copy of the toxin encoded at the C-terminal end (Woodward *et al*., 1990; Colledge *et al*., 1992). Peptide diversification appar-

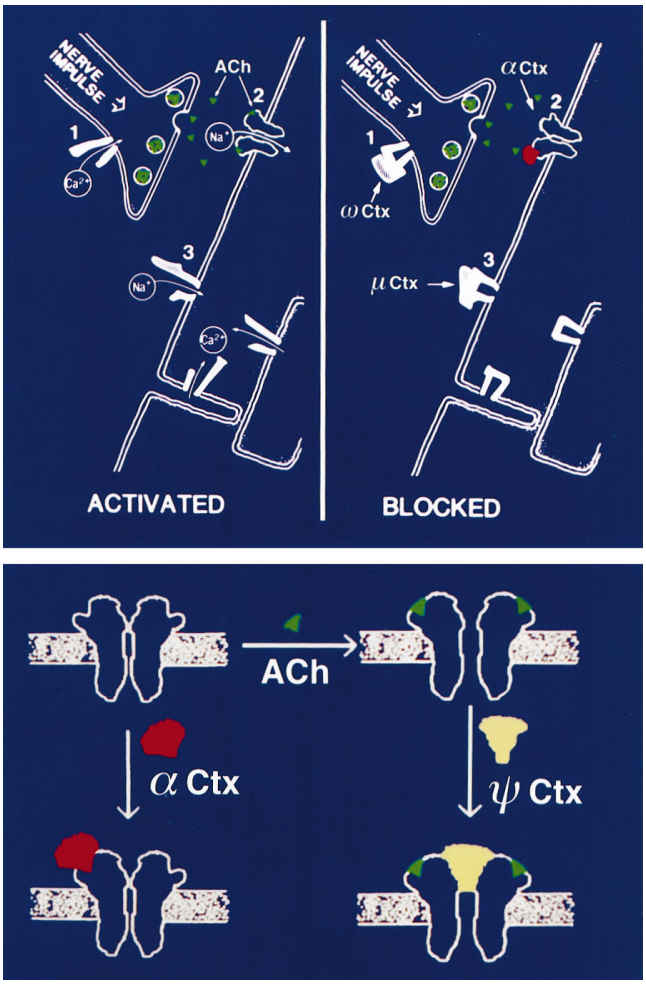

Figure 4. Mechanism of blocking neuromuscular transmission. The "motor cabal" of toxins (see text) targets multiple components in the neuromuscular circuit (top panel). Three different toxins (ω-, α- and μ-conotoxins) act on three different target components: 1) presynaptic Ca^{2+} channels; 2) nicotinic acetylcholine receptors; and 3) voltage-gated skeletal muscle Na^{+} channels. Bottom panel, synergy in *Conus* venoms is achieved both by inhibiting multiple components as well as multiple sites in a single macromolecular component. Two unrelated toxins (α- and Ψ-conotoxins) inhibit one receptor, the postsynaptic nicotinic acetylcholine receptor at different sites.

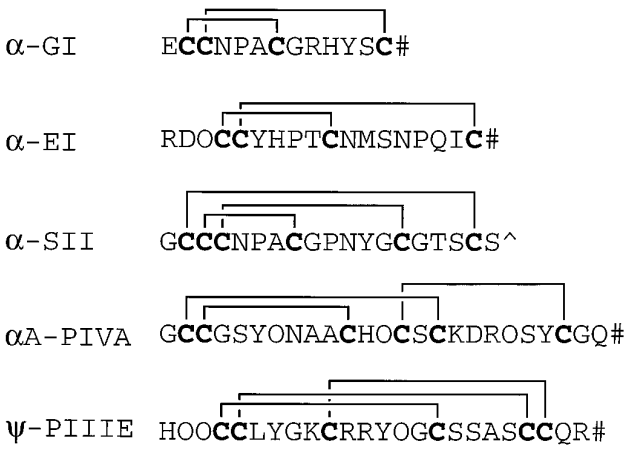

Figure 5. Diverse structures of peptides from different cone snails which inhibit the nicotinic acetylcholine receptor at the postsynaptic terminus of the neuromuscular junction. The first four compounds [α-GI (Gray *et al.*, 1981); α-EI (Martinez *et al.*, 1995); α-SII (Ramilo *et al.*, 1992); αA-PIVA (Hopkins *et al.*, 1995)] are all competitive antagonists of the receptor. The fifth peptide, Ψ-PIIIE (Shon *et al.*, 1997a) acts at a different site on the receptor complex and is a noncompetitive antagonist. Note the lack of sequence similarity between all of these peptides which are all produced in the venom ducts of fish-hunting *Conus* species. ^, free C-terminus; #, amidated e-terminus; O, hydroxyproline.

ently arises by focal hypermutation of the C-terminal toxin-encoding region while the rest of the precursor sequence remains largely conserved. The most conserved sequence feature of all is the signal sequence (Fig. 6).

In many ways, the pattern of conserved and variable regions in *Conus* peptide precursors is opposite that of conventional secreted polypeptides. Two *Conus* peptide precursors, ω-conotoxins MVIIA and GVIA (Olivera *et al.*, 1984, 1987) from two different fish-hunting cone snails, are compared in Fig. 6. These two ω-conotoxins target precisely the same site on an α_{1B} subunit of a voltage-gated calcium channel. Total sequence conservation might have been predicted for two such peptides that both target the same site and are found in species in the same genus. In contrast, we would not, *a priori*, expect signal sequence conservation (in most secreted proteins, the N-terminal signal region is the least conserved sequence element). The reality (Fig. 6) is the converse of these expectations: the signal sequence region is completely conserved, but the mature toxins show extreme divergence in sequence (≦30% of noncysteine amino acids conserved). This juxtaposition of conserved and hypervariable regions within the same translation product is reminiscent of antibody-encoding genes in the mammalian immune system, where special genetic mechanisms have evolved to generate diversity.

In the midst of the C-terminal toxin region which is generally hypervariable, the cysteine residues involved in the disulfide framework of the mature toxin are totally conserved. It is noteworthy that many conotoxin N-terminal signal sequences contain one or two cysteine residues (for example, those shown in Fig. 6), whereas the longer intervening propeptide region (between signal sequence and C-terminal mature toxin peptide) never has any Cys residues. The extremely conserved signal sequence may be optimally designed to target the precursor to a specific region in the endoplasmic reticulum; this may be important for specific posttranslational modifications as well as sorting into specialized secretory vesicles. In addition, how-

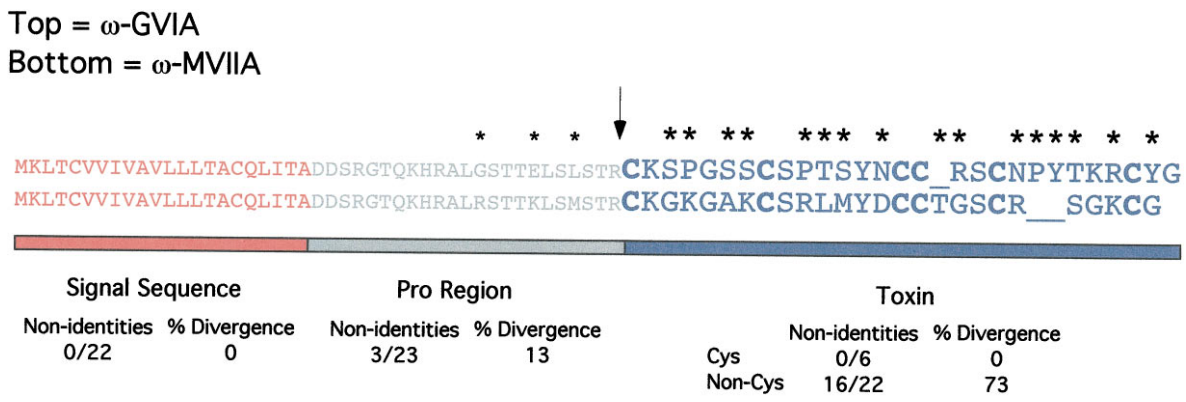

Figure 6. A comparison of the precursor sequences of two ω-conotoxins, GVIA (top sequence) and MVIIA (bottom sequence) from *Conus geographus* and *Conus magus*, respectively. Both of these toxins block the N-type voltage-gated calcium channel and compete with each other for binding. The entire precursor sequence is shown. The red amino acids comprise the signal sequence, whereas the solid arrow indicates the proteolytic site which generates the mature toxin. The mature toxin region is shown in capital letters, and the cysteine residues involved in disulfide bonding are shown bold. All loci where the two sequences differ are indicated by asterisks. It is notable that although the signal sequences are entirely identical, the vast majority of the non-Cys amino acids of mature ω-conotoxin GVIA are different from the corresponding ω-conotoxin MVIIA residues; in contrast, the Cys residues in this region are completely conserved.

ever, the presence of Cys residues in many signal sequences suggests that signal sequences may also play a sequestration role, i.e., to avoid premature or inappropriate disulfide bond formation in the mature toxin region. The latter hypothesis predicts that a mutation either in a Cys residue in the mature toxin region or in the signal sequence may perturb proper processing and secretion. Such mutations would be selected against at the cellular processing level, resulting in a coconservation of N-terminal signal sequences and Cys residues in the C-terminal toxin region. Thus, rapid toxin evolution may occur via a pathway in which conservation of structural frameworks based on Cys residues is selected for, even at the level of cellular processing of precursors.

Rapid hypermutation of venom peptides could be an optimum evolutionary strategy when prey, predators, and competitors change very rapidly (due to a sudden climate change or geological catastrophe, for example). Special mechanisms may have evolved that accelerate the generation of new venom peptides—in effect, by hypermutating the variable sequences between disulfide frameworks, cone snails employ a combinatorial library search strategy to evolve new peptides in their venoms.

Although hypermutation of peptide sequences is undoubtedly the main engine for generating peptide diversity in *Conus*, an unprecedented series of posttranslational modifications provide an overlying level of diversity (Jimenez *et al*., 1997). Some of these modifications (such as epimerization of L-tryptophan to D-tryptophan and posttranslational bromination of Trp to 6-Br-tryptophan) have not been described previously outside the *Conus* peptide system. Others (i.e., γ-carboxylation of glutamate to γ-carboxyglutamate) were described previously only in specialized, phylogenetically distant systems. One recently characterized small *Conus* peptide may be the most intensively posttranslationally modified polypeptide known: six posttranslational modifications occur in the mature toxin region to generate the final functional gene product of only eight amino acids (Jimenez *et al*., 1997). In some cases, posttranslational modification occurs to constrain *Conus* peptide conformation. For example, the posttranslational γ-carboxylation of glutamate residues (McIntosh *et al*., 1984) strongly promotes formation of an α-helix in at least some *Conus* peptides (Myers *et al*., 1990).

EVOLVING HIGHLY REFINED TARGETING

One important trend in modern drug development is to refine drug target specificity. Particularly for pharmacological agents that need to be applied chronically, severe side effects are a continuing problem. A current research imperative is to develop "clean drugs" that only target the therapeutically relevant molecule and not other closely related subtypes. In this respect, cone snails excel greatly compared to what the drug industry can produce at the present time. The *Conus* peptide system may help to reveal molecular recognition principles that underlie highly refined targeting.

In almost every case that has been carefully examined, *Conus* peptides appear to be much more selective than other ligands targeting the same receptor. Thus, the major paralytic α-conotoxin series in fish-hunting cone snail venoms (such as α-conotoxin GI shown in Fig. 5) discriminate between the two ligand-binding sites on the nicotinic acetylcholine receptor by three to

four orders of magnitude; in contrast, analogous snake toxins such as α-bungarotoxin have affinities that differ little for the two ligand-binding sites. Additionally, the snake toxins target certain neuronal nicotinic receptors (belonging to the α7 subtype)—these are not targeted at all by the α-conotoxins. Thus, this group of α-conotoxins may be the most specifically targeted nicotinic antagonists known. Another example of exceptionally selective targeting are the μ-conotoxins, which exhibit much more specificity for particular sodium channel subtypes (i.e., those of skeletal muscle) than do the classical guanidinium toxins, tetrodotoxin and saxitoxin.

Why are *Conus* peptides exceptionally selective for their receptor targets? Two reasons are suggested by cone snail biology. The necessity for very rapid prey immobilization would provide strong selection pressure for ligands not to bind physiologically irrelevant targets. Binding to other molecules with K_Ds < 0.1 μM could mean multiple rounds of association and dissociation en route to the true physiological receptors. This would cause a lag before the peptide could elicit its biological effect. In addition, increasing venom complexity raises the probability that two peptides in the same venom would jam each other's function physiologically, a possibility greatly diminished if venom peptides were narrowly targeted. In the work on *C. purpurascens* venom described above (Terlau *et al*., 1996), one peptide increases total inflow of sodium through voltage-gated sodium channels (part of the lightning strike cabal), where another peptide blocks conductance through sodium channels (part of the motor cabal). Clearly, the two peptides would act at cross purposes if they were to act on sodium channels of the same cells. In fact, each peptide is targeted with great specificity to different molecular forms of voltage-gated sodium channels located in distinct populations of cells. Consequently, in vivo, no functional interference occurs between these two potentially antagonistic venom components.

The specificity of *Conus* toxins can be very impressive indeed. In the case of voltage-gated Ca^{2+} channel-targeted toxins (such as ω-conotoxins GVIA or MVIIA) discrimination between various calcium channel subtypes can be $>10^6$-fold (Olivera *et al*., 1994). The ability to discriminate between closely related members of the calcium channel family is being exploited medically to develop one *Conus* peptide, ω-conotoxin MVIIA as a drug for the alleviation of intractable pain. Within the spinal cord, the targeted α_{1B}-containing voltage-gated calcium channel complexes are largely restricted to sensory regions. The high degree of target discrimination exhibited by this peptide has made it a feasible candidate for drug development; it is now in advanced clinical trials (Bowersox *et al*., 1997). In addition, a class of subtype-specific *N*-methyl-D-aspartate receptor antagonists, the conantokins, exhibit unique properties that give them considerable potential for development as anticonvulsant drugs (White *et al*., 1997).

A major question which needs to be investigated further are the mechanisms by which *Conus* peptides discriminate among target subtypes. Recently, evidence obtained by my colleagues J. Michael McIntosh, G. Edward Cartier, and Doju Yoshikami on a conotoxin whose structure was solved by Shon *et al*. (1997b) suggests a "dock-and-lock" recognition mechanism. The very high subtype selectivity of this *Conus* peptide appears to arise from the presence of two distinct interaction faces, called the "docking" and the "locking" faces, which in turn interact with complementary docking and locking sites on the target receptor. The experimental results are consistent with initial contact being made by the docking face on the peptide interacting with a docking site on the receptor; this then facilitates formation of functionally significant locking interactions of the toxin locking face with the receptor locking site.

We have coined the term "Janus-ligand" when, as in the example above, two interaction faces are present that are oriented in different directions (after Janus, the two-faced Roman god of beginnings). This could provide a basis for much more refined discrimination between homologous members of a large receptor or ion channel family. We suggest that many *Conus* peptides may be similarly two-faced Janus-ligands that use a strategically different molecular recognition paradigm from "normal" ligands. Other novel design strategies for subtype discrimination may well have evolved in these venoms.

CONE SNAIL PEPTIDES: AN HISTORICAL VIEW

A plausible scenario can be constructed for how cone snails may have evolved their complex venoms over the last 50 million years. Once the first venom able to cause prey paralysis by targeting a key component of the nervous system had evolved, a gradual expansion of the pharmacopoeia to yield more and more effective venom for prey immobilization may have taken place (this could well have occurred in taxa ancestral to *Conus*). Additionally, any venom components that could be used defensively would confer a strong selective advantage. As the venoms of *Conus* species slowly assumed greater complexity with time, these would then be subject to more selective variables.

With increasing venom complexity, a successive series of sudden changes in the environment would be expected to provide a correspondingly greater advantage to species that could mutate their venoms relatively quickly to adapt to a new ecological context, providing a "first-out-of-the-gate" evolutionary advantage. This is quite analogous to the observation

(LeClerc *et al*., 1996) that pathological microorganisms that have recently colonized new hosts have a much higher frequency of the mut phenotype, which confers a greatly increased frequency of mutation. Furthermore, when successive rounds of strong selection are applied in vitro, an entire bacterial population is found with the hypermutagenic mut phenotype (Mao *et al*., 1997). We suggest that successive extreme changes in climate and/or geological catastrophes may similarly select for hypermutation phenotypes in macroorganisms, with *Conus* peptides being an extreme example. The ability of cone snails to rapidly evolve a new complement of peptides after a geological catastrophe may be the key factor in the species richness of the genus *Conus* at the present time.

Our historical reconstruction predicts that for the last 50 million years peptides selected for one set of prey or predators may have had to be successively (and rapidly, in a geological time scale) mutagenized, thus generating a new set of peptides optimized to a different ecological context. However, receptors and ion channels in different nervous systems are quite conserved. Thus, a ligand for a nicotinic receptor targeted to emerging new prey would most likely be derived by mutagenesis from a gene encoding a ligand for nicotinic receptors in the original biological context, to take a specific example. Thus, a nicotinic receptor antagonist in a present-day cone snail venom may, in the course of its geological history, have undergone multiple rounds of selection for optimally targeting nicotinic receptors in different animals and even for different nicotinic receptor subtypes.

Thus, over tens of millions of years, the ligands present in *Conus* venoms may, in effect, have been subject to multiple rounds of selection against a target receptor family. Janus-ligands may be one consequence of such reiterative selection over evolutionary time. Much of the molecular diversity in all nervous systems stems from assembling macromolecular complexes in a modular and combinatorial fashion. Heteromeric complexes are characteristic of both ligand-gated ion channels (such as the nicotinic receptor) or voltage-gated channels (such as K^+ channels) with subunit interfaces of the type $\alpha x\beta y$ where αx and βy may represent members of two different gene families with many homologues ($\alpha 1$, $\alpha 2$, $\alpha 3$ . . . etc., and $\beta 1$, $\beta 2$, $\beta 3$, etc.). The functional receptor or ion channel complex is typically a tetrameric or pentameric combination of specific α and β subunits. Ordinary ligands would generally target any receptor complex which contained a high-affinity subunit target (such as αx). Janus-ligands with two different interaction faces would be one structural design adaptation that would be favored by reiterative rounds of selection for targets with a modular organization. Because a Janus-ligand has two distinct recognition interfaces, it would have much higher specificity (i.e., of the αx-containing complexes, only those specifically containing adjacent $\alpha x\beta y$ subunits would be high affinity targets); this would pick out a specific target isoform from many closely related ones in a large family of heteromeric receptors or ion channels. There is much that remains to be understood about the venom system and individual venom peptides observed in cone snails today; it seems wise to keep in mind that these are the end result of the historical forces described above.

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