Life Sciences 87 (2010) 451–456

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Minireview

Diselenium, instead of disulfide, bonded analogs of conotoxins: novel synthesis and pharmacotherapeutic potential

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ARTICLE INFO

Article history:
Received 19 May 2010
Accepted 20 July 2010

Keywords:
Cone snail
Conotoxin
Diselenide
Disulfide

ABSTRACT

The venoms of the cone snail (*Conus*) contain toxic peptides (conotoxins) that have remarkable selectivity for subtypes of a variety of mammalian voltage- and ligand-gated ion channels, G protein-coupled receptors, and neurotransmitter transporters. They thus have tremendous potential as pharmacologic tools. Less toxic analogs or mimetics could be highly-selective pharmacotherapeutic agents at their target sites. For this reason, conopeptides have been extensively studied and have progressed to clinical trials and even regulatory approval. However, the synthesis of the peptides remains difficult and stability and toxicity remain problems. A novel synthesis and testing of analogs incorporating diselenium bonds between selenocysteine residues in place of disulfide bonds between cysteine residues has recently been reported. The technique results in analogs that retain the folding of the native peptides, are more potent, and have the same or greater biological activity.

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Introduction

Conotoxins are members of a large family of highly potent peptide toxins (venoms), so named because they are released by the approximately 500 – 700 diverse species of the predatory marine cone snail genus *Conus* (Fig. 1A) found in all tropical marine habitats (e.g., Becker and Terlau 2008; Cruz 1996; Glaser and Mayer 2009; Gray et al. 1988; Heading 2004; Tsetlin et al. 2009; Yoshiba 1984). The conotoxins rapidly and effectively paralyze the slow-moving cone snail's prey for easier predation. The majority of conotoxins are thought to produce this effect by an action on membrane voltage-gated or ligand-gated ion channels, but other targets include GPCRs (G protein-coupled receptors) and neurotransmitter transporters (e.g., Castellino and Prorok 2000; Ekberg et al. 2008; Favreau et al. 1999; Hamilton and Perez 1987; Lewis 2004). These and related actions generalize to mammalian voltage- and ligand-gated ion channels and other neurotransmitter-related processes and provide

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doi:10.1016/j.lfs.2010.07.011

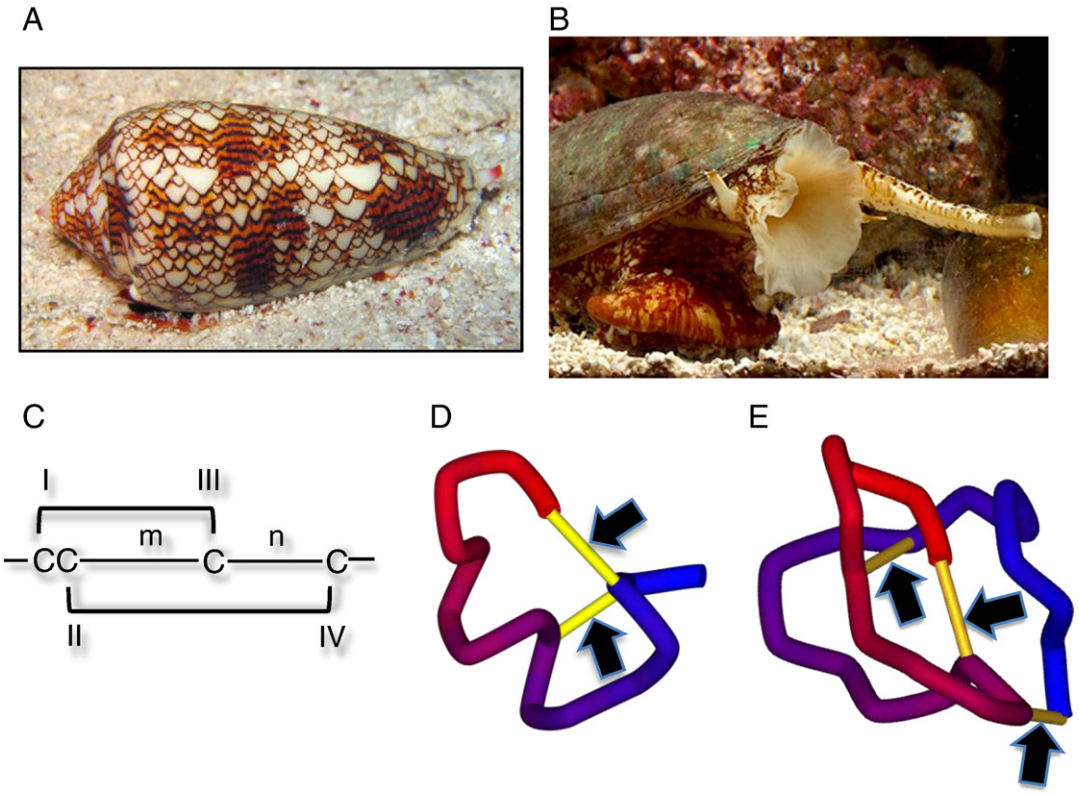

Fig. 1. A: *Conus textile*. B: *Conus geographus*. C: Disulfide bonds form between Cys residues with m and n intermediate amino acids. D: α-Conotoxin from PnIB. E: ω-Conotoxin MVIIA (ziconotide). The disulfide bonds in D and E are indicated by arrows.

the basis for current pharmacotherapy and potential for new drug development (e.g., Armishaw and Alewood 2005; Glaser and Mayer 2009; Twede et al. 2009; Watters 2005).

The mechanism of biological activity of conotoxins is highly dependent upon strict complementary structural determinants. Conotoxins are peptides ('conopeptides') commonly consisting of 10 – 30 amino acid residues (e.g., Craik and Adams 2007; Marx et al. 2006). However, they are not linear peptides; in addition to the usual thermodynamic impositions, typically one or more internal disulfide bonds impose folding constraints on their 3-dimensional structure (e.g., Bulaj and Olivera 2008; Walewska et al. 2008). 'Conotoxin' is the term commonly used when referring to disulfide-rich cone snail toxins; 'conopeptide' is a more general term used when referring to any peptide found in cone snail venom.

In a recent paper, Muttenthaler et al. (2010) report a facile method for synthesis and evaluation of conotoxin analogs that have diselenium bonds between selenocysteine rather than the native disulfide bonds between cysteine residues. These analogs adopt conformations similar to their disulfide counterparts and maintain, or in some cases exceed, their biological activity. As such, they offer promise as pharmacologic tools and also as possible therapeutic agents.

Toxicologic activity of conotoxins

When a cone snail's extended distensible proboscis contacts a prey, one or more harpoon-like, hollow, toxin-containing teeth are propelled out of the proboscis and injected into the grasped prey, which is held in contact by circular muscles at the anterior tip of the proboscis (Terlau and Olivera 2004). Depending on the species, toxin injection can be a single or repeated event and the detailed strategy for prey capture is also species-dependent (e.g., Kohn et al. 1960).

Terlau and Olivera (Terlau and Olivera 2004) describe cone snails as "sophisticated practitioners of combination drug therapy". This characterization is based on the fact that the snails release multiple peptides that act in a coordinated –– sometimes even synergistic –– fashion to produce the desired soporific or toxicologic effect on the injected prey, predator, or competitor. Such an assemblage of conopeptides, acting in a concerted manner to a specific physiological end point, has been termed a 'toxin cabal' (Terlau et al. 1996). Two broad mechanistic classes of toxin cabals, termed 'lightening-strike' and 'motor' have been differentiated (Terlau et al. 1996) and are summarized in Table 1. Note that the seemingly counteractive actions on Na^+ channels. This is avoided because each of the peptides is selectively targeted to specific and non-interfering different Na^+ channel subtypes. Other strategies have been termed 'false mouths' (net-like projections that hold the prey for injection) and 'nirvana cabal' that interrupt sensory circuits of the prey, making them quiescent and easier to capture (e.g., Olivera and Cruz 2001). Some cone snails contain toxin sufficient to kill a human (Yoshiba 1984).

Conotoxin subtypes

Each of the estimated 500 different species of cone *Conus* synthesizes and injects its own 'repertoire' of about 100 peptide toxins in their venom, giving rise to about 50,000 biologically active compounds. The peptide toxins are initially translated as prepropeptide precursors. Proteolytic cleavage and extensive posttranslational modification yields the diversity of final products (Olivera 1997). In addition to peptides, the venom of a small number of species also contains serotonin (5-HT), arachidonic acid, polypeptides, or enzymes (e.g., Lirazan et al. 2002; McIntosh et al. 1995).

For purposes of correlation to their biological activity, it is convenient to categorize conotoxins according to their physiologic actions on biological tissues. The majority of known components of conotoxins target specific voltage-gated and ligand-gated ion channels. Other known toxin components target GPCRs (Craig et al. 1998; Cruz et al. 1987; Sharpe et al. 2001) and possibly Ca^{2+} channels (Rigby et al. 1999; Walker et al. 1999), or norepinephrine transmitter (Sharpe et al. 2001). Widely conserved peptide families target voltage-gated Na^+-channels (both TTX-sensitive and TTX-insensitive types), including: μ -conotoxins