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# Structure, function and therapeutic potential of omega conopeptides: Novel blockers of neuronal calcium channels+

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**Abstract.** Recent molecular cloning strategies have revealed that the diversity of voltage-sensitive calcium channels (VSCC) in the nervous system is much larger than indicated by electrophysiological studies. Elucidation of the physiological functions of these novel classes of VSCC requires pharmacological tools such as the blockers which were highly useful in characterizing the L-type VSCC in skeletal, smooth and cardiac muscle. Omega conopeptides found in the venom of fish-eating marine snails are proving to be highly selective blockers of neuronal VSCC. Several omega conopeptides have been isolated from a variety of *Conus*, characterized and synthesized. Biochemical, electrophysiological and morphological studies with these synthetic conopeptides have shown that novel types of VSCC are located in discrete regions of the brain and control the release of neurotransmitters in a highly selective manner, hitherto unsuspected. Pharmacological studies in animal models of cerebral ischemia have shown that omega conopeptides which selectively block N-type VSCC are highly effective in preventing brain damage caused by the loss of oxygen supply to the brain during the ischemia episode.

**Keywords.** Omega conopeptides; neuronal calcium channels; *Conus* species.

## Introduction

Marine snails of the genus *Conus* are a rich source of peptides that display high affinity and specificity for several targets in the central nervous system (Olivera *et al* 1991). Among these, the omega conopeptides derived from *Conus geographus* and *Conus magus* have emerged as valuable tools for characterizing neuronal voltage sensitive calcium channels (VSCC). The N-type VSCC which mediate a substantial portion of the calcium-mediated neurotransmitter release at presynaptic nerve terminals, have been distinguished from other types of calcium channels by their sensitivity to the omega conopeptide GVIA from *Conus geographus* (McCleskey *et al* 1987). Another omega conopeptide, MVIIA, from *Conus magus* has been found to be a more selective and reversible blocker of N-type VSCC (Olivera *et al* 1987; Kristipati *et al* 1994) whereas MVIIC also derived from *Conus magus* displays a selectivity for neuronal VSCC that is different from both GVIA and MVII (Hillyard *et al* 1992; Kristipati *et al* 1994). This peptide MVIIC was found to be an effective inhibitor of GVIA resistant synaptic transmission and P-type VSCC which have been

+This article is dedicated to Professor CNR Rao, eminent scientist, scholar and educator, on the occasion of his sixtieth birthday

described in cerebellar Purkinje cells, in addition to its effects on N-type VSCC (Hillyard *et al* 1992). In view of the larger diversity of VSCC that is emerging from molecular cloning strategies (Tsien *et al* 1991) investigation of the omega conopeptides from other species of *Conus* is likely to be highly rewarding. The structure and function of the two synthetic peptides SNX-111 (MVIIA) and SNX-230 (MVIIC) are reviewed in this article.

## Structural features

The amino acid sequences of SNX-111 (MVIIA) and SNX-230 (MVIIC) are compared in table 1. Both contain six cysteine residues arranged into three disulphide bridges. The disulphide bonding in both peptides have been shown to be the same, namely, the first cysteine to the fourth, the second to the fifth and the third to the sixth. There is considerable sequence similarity between the two peptides in the amino and carboxy terminal segments. Seven out of the first eight residues and eleven of the fourteen carboxy terminal segments are identical. The major difference between the peptides lies in the middle segment (residues 9–12) where only one residue is common to the two peptides. SNX-111 and SNX-230 were prepared by solid phase peptide synthesis and characterized (Hillyard *et al* 1992; Valentino *et al* 1993). SNX-183, corresponding to the conopeptide SVIB from *Conus striatus* (Ramilo *et al* 1992), bears close resemblance to SNX-230.

### *Conopeptides distinguish neuronal VSCC subtypes*

The presence of a single tyrosine residue in position 13 in both SNX-111 and SNX-230 enabled the preparation of $^{125}$I-labelled peptides which could be used to characterize binding sites in rat brain synaptosomal preparations (Kristipati *et al* 1994). Saturation binding analysis revealed a single high affinity site for $^{125}$I-SNX-111 with an apparent dissociation constant (*Kd*) of 0·009 nM. Analysis of the displacement of $^{125}$I-SNX-111 with unlabelled SNX-111 showed that SNX-111 is more potent than the iodinated compound in its affinity for this binding site which corresponds to N-type VSCC (*Ki* 0·001 nM; Kristipati *et al* 1994). SNX-230 on the other hand, displaced $^{125}$I-SNX-111 with a lower affinity (*Ki* 0·086 nM). Similar saturation binding and displacement studies using $^{125}$I-SNX-230 (table 2) revealed a high affinity site for SNX-230 that is distinct from the N-type VSCC recognized by SNX-111. Although saturation binding

**Table 1.** Natural ω-conopeptide sequences. SNX numbers refer to the synthetic peptides prepared and characterized at Neurex.

| Name | Sequence[a] | Species |
|---|---|---|
| SNX-111 (MVIIA) | CKGKGAKCSRLMYDCCTGSC-R-SGKC | *C. magus* |
| SNX-230 (MVIIC)[b] | CKGKGAXCRKTMYDCCSGSCGR-RGKC | *C. magus* |
| SNX-183 (SVIB) | CKLKGQSCRKTSYDCCSGSCGR-SGKC | *C. striatus* |

[a]All synthetic peptides are amidated at the carboxy terminus

[b]The sequence corresponding to SNX-230 is deduced from the cDNA sequence of a clone isolated from *C. magus* cDNA library (Hillyard *et al* 1992).

**Table 2.** Selectivities of conopeptides for site 1 and site 2[a].

| Compound | *Ki*(nM) for competition with | | Selectivity for site 1:site 2 |
|---|---|---|---|
| | $^{125}$I-SNX-111 | $^{125}$I-SNX-230 | |
| SNX-111 | 0·001 | 135 | 135,000:1 |
| SNX-230 | 0·086 | 0·012 | 1:7 |

[a]*Ki* values were calculated from the results of competition binding experiments as described (Kristipati *et al* 1994). Selectivities are expressed as the ratio of the *Ki*'s for the two sites

with $^{125}$I-SNX-230 revealed a single class of high affinity sites with a *Kd* of 0·011 nM, displacement analysis showed that SNX-230 recognized this site with much higher affinity (*Ki* 0·012 nM) compared to SNX-111 (Ki 135 nM). These binding studies demonstrate that SNX-111 has extraordinarily high selectivity for N-type VSCC (site 1), namely, 135,000-fold over the novel type VSCC (site 2) recognized by SNX-230 (table 2). SNX-230 has a seven-fold higher affinity for this novel VSCC compared to N-type VSCC.

That the binding components recognized by SNX-111 and SNX-230 in the synaptosomomal preparations represent functional presynaptic calcium channels was confirmed by examining the effects of these two conopeptides on evoked neurotransmitter release (Ramachandran *et al* 1993). SNX-111 inhibited the release of norepinephrine evoked by potassium depolarization in rat hippocampal slices with high potency ($IC_{50}$ 1 nM) but only partially (~ 60%). SNX-230 inhibited the release completely but in a biphasic manner, inhibiting approximately 50% with high potency ($IC_{50}$ 0·02 nM) and 50% with much lower potency ($IC_{50}$ 65 nM). These results suggest that norepinephrine release is mediated by at least two distinct types of presynaptic calcium channels, one of which corresponds to the N-type VSCC (site 1) recognized by SNX-111 and with lower affinity by SNX-230, and the other to the novel type VSCC (site 2) recognized preferentially by SNX-230 only.

Autoradiographic studies of the distribution of the binding sites recognized by $^{125}$I-SNX-111 and $^{125}$I-SNX-230 also confirmed that these compounds recognize distinct types of VSCC located in discrete regions of the brain (Ramachandran *et al* 1993). Thus, high densities of binding sites for $^{125}$I-SNX-111 were found in the cortex, CA1, dentate gyrus, globus pallidus, CA2 and substantia nigra. $^{125}$I-SNX-230, on the other hand, decorated heavily the molecular layer as well as Purkinje cells and granule cells of the cerebellum, regions which were hardly recognized by $^{125}$I-SNX-111.

### *Middle segment of conopeptides confers selectivity*

As pointed out earlier, the major difference in the structures of SNX-111 and SNX-230 lies in the middle segment consisting of residues 9–12. Both SNX-111 and SNX-230 have relatively high affinity for N-type VSCC (site 1) but only SNX-111 displays high selectivity. Another omega conopeptide derived from *Conus striatus* called SVIB, the synthetic version of which is designated SNX-183, also differs from SNX-111 primarily in the middle segment (table 1; Ramilo *et al* 1992; Ramachandran *et al* 1993). SNX-183 recognizes both site 1 and site 2 with comparable affinity but much

**Table 3.** Influence of the middle segment of $\omega$-conopeptides on selectivity and transmitter release.

| Peptide | Middle segment sequence (residues 9–12) | $IC_{50}$(nM) for inhibition of binding of [$^{125}$I]-SNX-111 | $IC_{50}$(nM) for inhibition of binding of [$^{125}$I]-SNX-183 | Selectivity | $IC_{50}$(nM) for inhibition of norepinephrine release |
|---|---|---|---|---|---|
| SNX-111 | SRLM | 0·01 | 94 | 9400 | 1 |
| SNX-183 | RKTS | 1·70 | 4 | 2·3 | 180 |
| SNX-202[a] | SRLM | 0·05 | 16 | 320 | 15 |

[a]SNX-202 is a hybrid peptide containing amino acids 1 to 8 and 13 to 25 from XNX-183 and residues 9 to 12 from SNX-111. Selectivity values were obtained by dividing the $IC_{50}$ for competing with [$^{125}$I]-SNX-183 by the $IC_{50}$ for competing with [$^{125}$I]-SNX-111.

lower compared to SNX-111 or SNX-230 (table 3; Ramachandran *et al* 1993). Thus, the role of the middle segment of SNX-111 in recognizing N-type VSCC could be assessed by introducing residues 9–12 from SNX-111 in place of the corresponding residues in SNX-183. The hybrid peptide SNX-202 was found to bind to N-type VSCC (site 1) with thirty-fold higher affinity than SNX-183 and four-fold lower affinity to site 2 (table 3). The introduction of the middle segment of SNX-111 into SNX-183 converts the latter from a non-selective antagonist to one with a 320-fold selectivity for site 1 over site 2. The higher affinity of SNX-202 for N-type VSCC (site 1) is also reflected in its ability to block transmitter release whereas SNX-183 is 180 times less potent than SNX-111 in blocking norepinephrine release, SNX-202 is only 15 times less potent (Ramachandran *et al* 1993). These results imply that the middle segment of SNX-111 representing residues 9–12 is a major but not the sole determinant of the specificity of interaction of omega conopeptides with presynaptic calcium channel subtypes.

*Therapeutic potential of omega conopeptides*

The high potency and specificity of omega conopeptides in blocking neurotransmitter release mediated by presynaptic calcium channels make them attractive candidates for treating various pathological conditions in which neuronal calcium channels are involved. In view of its high selectivity in blocking N-type VSCC, SNX-111 has been evaluated in animal models of global ischemia where lack of blood supply to the brain results in damage to hippocampal and cortical neurons. Valentino *et al* (1993) found that SNX-111 protects the pyramidal neurons of the CA1 subfield of the hippocampus from damage caused by transient forebrain ischemia in rats. Furthermore, this neuroprotective effect of SNX-111 was observed even when the peptide was administered intravenously 24 h after the ischemic episode. On the other hand, SNX-230 which lacks the selectivity of SNX-111 was not effective in protecting against ischemic damage. These results suggest that the window of opportunity for therapeutic intervention after cerebral ischemia may be much longer than previously thought and point to the potential use of omega conopeptides with high selectivity for N-type VSCC in the prevention or reduction of neuronal damage resulting from ischemic episodes due to cardiac arrest, head trauma or stroke.

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