Conotoxins targeting voltage-gated sodium channels: harnessing nature's analgesics

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μ-Conotoxins are a family of peptides from predatory marine cone snails that target voltage-gated sodium channels (VGSCs), blocking the passage of sodium ions through the channel. Several neuronal VGSC subtypes have been implicated in the perception of pain; as such, modulators of these subtypes could have potential therapeutic use as analgesics. μ-KIIIA shows potent analgesic activity following its systemic administration in mice (Zhang *et al.*, 2007). Structure-activity studies indicated that the key residues important for VGSC-blocking activity (K7, W8, R10, D11, H12, R14) mostly resided on an α-helical motif and that a disulfide bond could be removed without significant loss of activity (Khoo *et al.*, 2009; Han *et al.*, 2009). These findings suggested a route for structural minimization of μ-KIIIA by retaining the key residues on an α-helical scaffold.

In stabilizing α-helices, the use of (*i*, *i*+4) lactam bridges has proven to be a successful approach. For a mimetic of μ-KIIIA, the result that Cys9 can be replaced with no significant loss in activity identifies a position in the helix that can be substituted to form a helix stabilizing (*i*, *i*+4) lactam bridge to either residue 5 or 13, both of which are non-essential residues and therefore replaceable. We have designed and synthesized several analogues of μ-KIIIA; all of them are truncated at both the N- and C-termini, and the remaining sequence is stabilized by a lactam bridge at strategic locations. The helicity of six lactam analogues has been analysed using NMR spectroscopy and molecular modelling, and their activities have been tested against a range of VGSC subtypes (Khoo *et al.*, 2011). Our findings highlight important structure-activity relationships and provide a basis for the design of new minimized peptides and helical mimetics as novel analgesics.

In the course of our studies on μ-KIIIA it became apparent that the major product from oxidative refolding adopts a {1-15,2-9,4-16} disulfide pattern rather than the {1-9,2-15,4-16} disulfide pattern predicted on the basis of closely-related μ-conotoxins (Khoo *et al.*, 2009). Moreover, a minor product adopts a {1-16,2-9,4-15} pattern. Surprisingly, both products are capable of blocking the channel (Khoo *et al.*, 2012), highlighting the fact that different structures can present the key functional groups. The difficulties in defining the disulfide connectivities of the products of oxidative refolding *in vitro* further emphasize the value of alternative strategies such as those pursued here for stabilising peptide structures.

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