Conotoxins targeting voltage-gated sodium channels: Designing new analgesics

R.S. Norton,[1,2] K.K. Khoo,[1,2,3] Z. Kuang,[2] B.J. Smith,[2] M.J. Wilson,[4] M-M. Zhang,[4] J.E.F. Rivier,[5] D. Yoshikami,[4] B.M. Olivera[4] and G. Bulaj,[4,6] [1]Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, VIC 3052, Australia, [2]The Walter & Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, VIC 3052, Australia, [3]The Department of Medical Biology, University of Melbourne, Parkville, VIC 3010, Australia, [4]Department of Biology, University of Utah, Salt Lake City, Utah 84112, USA, [5]Salk Institute for Biological Studies, La Jolla, CA 92037, USA and [6]Department of Medicinal Chemistry, College of Pharmacy, University of Utah, Salt Lake City, Utah 84108, USA.

μ-Conotoxins are a group of toxins 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 of VGSCs could have potential therapeutic use as analgesics. μ-conotoxin KIIIA (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 alpha-helical motif and that the first disulfide bond could be removed without significant loss of activity (Khoo *et al.*, 2009). These findings suggested a route for 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 generates 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 are replaceable. We have designed and synthesized several analogues of μ-KIIIA; all of them are truncated at both N- and C-terminal ends, and the remaining sequence is stabilized by a lactam bridge at strategic locations. The helicity of the six lactam analogues has been analysed using NMR spectroscopy and molecular modelling, and their activities have been tested against a range of VGSC subtypes. Our findings highlight important structure-activity relationships and provide a basis for the design of new minimized peptides and helical mimetics as novel analgesics.

Zhang MM, Green BR, Catlin P, Fiedler B, Azam L, Chadwick A, Terlau H, McArthur JR, French RJ, Gulyas J, Rivier JE, Smith BJ, Norton RS, Olivera BM, Yoshikami D, Bulaj G. (2007) *J Biol Chem* **282,** 30699-30706.

Khoo KK, Feng ZP, Smith BJ, Zhang MM, Yoshikami D, Olivera BM, Bulaj G, Norton RS. (2009) *Biochemistry* **48,** 1210-1219.