Biological activity of alanine-substituted analogues of α-conotoxin Vc1.1 on N-type calcium channels in rat sensory neurons

B.P. Callaghan,[1] J. Jensen,[2] R.J. Clark,[2] D.J. Craik[2] and D.J. Adams,[1] [1]Health Innovations Research Institute, RMIT University, Bundoora, VIC 3083, Australia and [2]Institute for Molecular Biosciences, University of Queensland, St Lucia, QLD 4072, Australia.

α-Conotoxin Vc1.1 is a 16 amino acid disulfide peptide that is a selective antagonist of the α9α10 nicotinic acetylcholine receptor (nAChR) subtype but has recently been shown to be a more potent inhibitor of N-type Ca^{2+} channel currents in dissociated neurons from rat dorsal root ganglia (DRG). The inhibition of N-type Ca^{2+} channel currents was blocked by inhibitors of $G_{i/o}$ and selective $GABA_B$ receptor antagonists suggesting that Vc1.1 acted *via* $GABA_B$ receptors (Callaghan *et al.*, 2008). To further explore the structure-activity relationship for Vc1.1 inhibition of N-type Ca^{2+} channels in DRG neurons, the amino acids except the conserved cysteines contained in the sequence of Vc1.1 (Gly(1)-Ser(4)-Asp(5)-Pro(6)-Arg(7)-Asn(9)-Tyr(10)-Asp(11)-His(12)-Pro(13)-Glu(14)-Ile(15)), were sequentially replaced by Ala. These analogues have been characterised by NMR spectroscopy demonstrating that the structure of the peptide is not significantly changed (Halai *et al.*, 2009). The present study examined the activity of the Vc1.1 analogues on high voltage-activated Ca^{2+} channel currents in rat DRG neurons using the whole-cell patch clamp technique. Analogues that resulted in significant shifts to the right of the concentration-response relationship for inhibition of Ca^{2+} channel currents included S4A (n=4), N9A (n=16) and P13A (n=2). In contrast, analogues with the least effect or unchanged compared to Vc1.1 were D11A (n=4), E14A (n=6) and I15A (n=3). Interestingly [N9A]Vc1.1 has been reported to be more potent than Vc1.1 at the α9α10 nAChR whereas it is inactive at inhibiting N-type Ca^{2+} channel currents. These findings contribute to an improved understanding of the molecular basis for the $GABA_B$ receptor-mediated inhibition of the N-type calcium channel current by Vc1.1.

Callaghan B, Haythornthwaite A, Berecki G, Clark RJ, Craik DJ, Adams DJ. (2008) *J. Neurosci.* **28**: 10943-51.
Halai R, Clark RJ, Nevin ST, Jensen JE, Adams DJ, Craik DJ. (2009) *J. Biol. Chem.* **284**: 20275-84.