Analgesic conotoxins: modulation of voltage-gated calcium channels in pain pathways

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The small and highly structured peptides found in the venom of marine cone snails target a wide variety of membrane receptors and ion channels in normal and diseased states. A number of these peptides (conotoxins) have shown efficacy *in vivo* including inhibitors of voltage-gated sodium (Na_v) and calcium (Ca_v) channels and nicotinic acetylcholine receptors (nAChRs) which are in preclinical development for the treatment of chronic and neuropathic pain. A number of structurally related ω-conotoxins bind directly to and selectively inhibit N-type calcium channels of nociceptive dorsal root ganglion (DRG) neurons. Among these, ω-conotoxin MVIIA (Prialt) still maintains its orphan drug status as a valuable alternative intrathecal analgesic for the management of chronic intractable pain, especially in patients refractory to opioids. Newly discovered ω-conotoxins from *Conus catus* are more potent and selective for N-type ($Ca_v2.2$) calcium channels over other Ca_vs (Berecki *et al.*, 2010). Furthermore, in spinal cord slices, these peptides reversibly inhibited excitatory monosynaptic transmission between primary afferents and dorsal horn superficial lamina neurons. In the rat partial sciatic nerve ligation model of neuropathic pain, ω-conotoxins CVIE and CVIF significantly reduced allodynic behaviour. Another family of conotoxins, the α-conotoxins, competitively inhibit nAChRs and bind at the interface between specific subunits allowing them to discriminate among different nAChR subtypes. α-Conotoxins Vc1.1 (ACV1) and RgIA are small disulfide bonded peptides currently in development as a treatment for neuropathic pain (Vincler *et al.*, 2006). It was proposed that the primary target of Vc1.1 and RgIA is the α9α10 neuronal nAChRs. Surprisingly, however, we found that Vc1.1 and RgIA more potently inhibit the N-type ($Ca_v2.2$) Ca^{2+} channel currents in rat sensory neurons *via* a voltage-independent mechanism involving the G protein-coupled $GABA_B$ receptor ($GABA_BR$) (Callaghan *et al.*, 2008). This was the first demonstration of α-conotoxins acting *via* the G protein-coupled $GABA_BR$ modulating native $Ca_v2.2$ channels. Recent molecular studies confirm that Vc1.1 and RgIA inhibit N-type Ca^{2+} channels *via* $GABA_BR$ activation. Transient transfection of DRG neurons with small interfering RNAs (siRNAs) to knock-down the $GABA_BR$ reduced mRNA levels for $GABA_B$ subunits by >50% compared to control cells and suppressed $GABA_BR$ protein expression. Whole-cell patch clamp recording of DRG neurons conducted 1-3 days after transfection demonstrated that knockdown of functional $GABA_BR$ expression significantly reduced the inhibition of N-type Ca^{2+} channels in response to both baclofen and Vc1.1. This was in contrast to neurons transfected with a non-targeting siRNA which were indistinguishable from untransfected neurons, confirming that α-conotoxin Vc1.1 modulates N-type Ca^{2+} channels *via* activation of $GABA_BR$ in DRG neurons. Our current findings have the potential to introduce a paradigm shift in thinking about the targets of α-conotoxins. $GABA_BR$ may play a critical role in pain pathways and are a clear therapeutic target for these and modified conotoxins.

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