Are α9α10 nicotinic acetylcholine receptors a pain target?

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The synthetic α-conotoxin Vc1.1 (ACV1) is a small disulfide bonded peptide currently in development as a treatment for neuropathic pain. Unlike Vc1.1, the native post-translationally-modified peptide vc1a does not act as an analgesic *in vivo* in rat models of neuropathic pain. Recently, it has been proposed that the primary target of Vc1.1 is the α9α10 nicotinic acetylcholine receptor (nAChR) (Vincler & McIntosh, 2006). The aim of the present study was to examine the potency and efficacy of the post-translationally modified analogues vc1a, [P6O]Vc1.1 and [E14γ]Vc1.1 at α9α10 nAChRs and in neuropathic pain studies, respectively.

Electrophysiological recordings from nAChRs exogenously expressed in *Xenopus* oocytes were as described previously . Membrane currents were recorded using an automated OpusXpress™ 6000A workstation. Acetylcholine (30 μM) was applied for 2 s with 400 s washout periods between applications. Conopeptides were bath applied and co-applied with the agonist. Cells were voltage clamped at –80 mV with peak current amplitudes measured before and following incubation of the peptide. Neuropathic pain was assessed using partial ligation of the left sciatic nerve (PNL) , with the effects of the conotoxins on withdrawal thresholds and motor function evaluated.

Vc1.1 has been shown previously to inhibit α3-containing nAChRs but only at micromolar concentrations and was inactive at concentrations up to 10 μM at α7, α4-containing and muscle (α1β1γδ) nAChRs expressed in oocytes (Clark *et al.*, 2006). Vc1.1 inhibited reversibly α9α10 nAChR-mediated currents in a concentration-dependent manner with an IC_{50} of 64.2 ± 15.0 nM (n = 12). Application of vc1a, [P6O]Vc1.1 and [E14γ]Vc1.1 also inhibited reversibly α9α10 nAChRs in a concentration-dependent manner, giving IC_{50}'s of 62.9 ± 5.2 nM, 99.1 ± 29.7 nM, 65.3 ± 14.9 nM (n = 10-12), respectively.

PNL produced a profound reduction in paw withdrawal threshold from a pre-surgery baseline of 12.9 ± 0.7 g to 0.7 ± 0.1 g (n = 33) 12-16 days after surgery. As reported previously (Satkunanathan *et al.*, 2005), intramuscular injection of 60 μg Vc1.1 produced significant partial reversal of allodynia associated with nerve injury. By contrast, 60 μg/rat injections of vc1a or [P6O]Vc1.1 had no significant effect on mechanical allodynia.

We demonstrate here that Vc1.1 is approximately 100-fold more potent for α9α10 nAChRs, and produces a significant partial reversal of allodynia associated with nerve injury. Similarly, the post-translationally modified peptides vc1a, [P6O]Vc1.1 and [E14γ]Vc1.1 inhibit α9α10 nAChRs with equivalent potencies to Vc1.1 and had no effect on mechanical allodynia in a nerve injury model of neuropathic pain. The lack of activity of vc1a on these nAChR subtypes is consistent with findings reported previously in bovine chromaffin cells and other rat models of neuropathic pain , however, vc1a is equally potent with Vc1.1 as an antagonist of α9α10 nAChRs. Synthetic vc1a and the partially modified homologues [P6O]Vc1.1 and [E14γ]Vc1.1 are all active at α9α10 nAChRs, but not at any of the other nAChR subtypes. However, given that Vc1.1, but not vc1a nor its analogue [P6O]Vc1.1, were able to inhibit a vascular response to pain and reduce chronic pain in several animal models of human neuropathy it is highly unlikely that the molecular mechanism or the therapeutic target for the treatment of neuropathic pain is *via* a α9α10 nAChR.

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