Biological activity of α-conotoxins on N-type calcium channels in rat sensory neurons

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Conotoxins are selective antagonists of a range of membrane receptors, ion channels and transporters associated with pain pathways. Previous studies have demonstrated the analgesic potential of several different α-conotoxins that competitively inhibit nAChRs with varying degrees of subtype selectivity. We have previously reported that the α9α10 nAChR-selective α-conotoxins Vc1.1 and RgIA inhibit N-type calcium channels in rat sensory neurons *via* a $GABA_B$ receptor-dependent signalling mechanism, which may contribute to their pain relieving actions (Callaghan *et al.*, 2008). We have also recently shown that the synthetic cyclisation of Vc1.1 produced an orally active peptide by improving *in vivo* stability (Clark *et al.*, 2010). Here we report that α-conotoxin AuIB, a weak but selective α3β4 nAChR antagonist, also inhibits high voltage-activated (HVA) Ca^{2+} channels in DRG neurons *via* a $GABA_B$ receptor dependent pathway whereas MII, a potent α3β2 nAChR antagonist does not. Native AuIB peptide found in the venom, has C1-C3, C2-C4 cystine globular connectivity whereas when AuIB is synthesized chemically, both the the globular and ribbon (C1-C4, C2-C3 connectivity) isomers are formed. The ribbon isomer of AuIB was also examined on $GABA_B$ receptor /N-type Ca^{2+} channel. α-Conotoxins were assessed on Ca^{2+} channel currents in rat DRG neurons using the whole-cell patch clamp recording technique. AuIB (100 nM) reduced peak Ca^{2+} channel current amplitude to 64.6 ± 6.2% of control with an IC50 of 1.5 ± 0.3 nM (n = 17). Application of the ribbon isomer of AuIB (100 nM) did not affect Ca^{2+} channel current amplitude (92.9 ± 3% of control, n = 5), whereas this isomer inhibits α3β4 nAChRs (Grishan *et al.*, 2010). Application of the selective N-type Ca^{2+} channel inhibitor, ω-conotoxin CVID, confirmed that AuIB targets the N-type component of the HVA Ca^{2+} channel currents. Preincubation with the receptor antagonist CGP 55845, blocked the effect of AuIB on HVA Ca^{2+} channel currents. MII at concentrations up to 1 μM did not inhibit depolarization-activated Ca^{2+} channel currents. The linker length of cyclised AuIB on Ca^{2+} channel current inhibition was also examined. Cyclised AuIB (100 nM) with 4 linking residues (GGAA) reduced Ca^{2+} channel current amplitude to 49.3± 8.3% of control with an IC50 of 5.9 ± 0.5 nM (n = 4) and cyclised AuIB (100 nM) with 5 linking residues (AGAGA) reduced Ca^{2+} channel current amplitude to 69.1± 8.8% of control with an IC50 of 21.3 ± 0.8 nM (n = 4). These findings demonstrate that α-conotoxins other than α9α10 nAChR-selective conotoxins inhibit N-type calcium channel currents *via* the $GABA_B$-mediated pathway and cyclisation of the peptide retains this inhibitory activity.

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