AuPS/ASB Meeting - Adelaide 2010

Symposium: Ion channel modulation by peptide toxins

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Chair: Ray Norton & David Adams

Probing the interaction between psalmotoxin 1 and acid sensing ion channel 1a, an analgesic drug target

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Acid sensing ion channel 1a (ASIC1a) is one of the primary acid sensors in the peripheral and central nervous system, and it has emerged as a novel target for the development of drugs to treat chronic pain, neurodegeneration, and possibly depression (Sluka *et al.*, 2009).The only known selective inhibitor of ASIC1a is psalmotoxin-1 (PcTx1), a 40-residue disulfide-rich peptide isolated from the venom of the Trinidad chevron tarantula *Psalmopoeus cambridgei*. PcTx1 is a potent blocker of ASIC1a (IC_{50} ~0.5nM) but it does not inhibit other ASIC subtypes (Escoubas *et al.*, 2000). Remarkably, PcTx1 has analgesic activity comparable to morphine in rat models of acute pain (Mazzuca *et al.*, 2007). With a view to using PcTx1 as a lead for development of novel analgesics, we developed an efficient bacterial expression system for production of recombinant toxin and determined a high-precision structure using 3D/4D triple resonance NMR spectroscopy. Site-directed mutagenesis revealed a highly cationic pharmacophore located within one of the intercystine loops. Molecular dynamics simulations in combination with NMR spin relaxation and relaxation dispersion measurements revealed significant motion in this loop over a wide range of timescales (ps to ms), thus precluding the use of rigid body docking protocols for modelling the toxin:channel complex. Instead, we used HADDOCK (Dominguez *et al.*, 2003) to dock the toxin onto a homology model of rat ASIC1a (rASIC1a). Key interacting residues identified from mutagenesis of the toxin and the channel were used as ambiguous interaction restraints and the sidechains of residues at the interaction interface were allowed to move during simulated annealing and refinement. The resulting model of the PcTx1:rASIC1a complex reveals a novel mode of interaction dominated by ion pair interactions involving arginine residues in the β-hairpin loop containing the toxin pharmacophore. The toxin:channel model is currently being used for *in silico* screening of chemical libraries to find nonpeptide mimetics of PcTx1.

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Sluka KA, Winter OC & Wemmie JA (2009) *Current Opinion in Drug Discovery & Development* **12**: 693-704.

Conotoxins targeting voltage-gated sodium channels: Designing new analgesics

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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.

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Subverting the biological actions of Conus peptides to modulate physiological responses

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The peptides from various *Conus* venoms have been grouped by Olivera (1997) into cabals, whose members have synergistic actions even though members of a single cabal may have different targets. For example, members of the motor cabal may inhibit muscle contraction and induce flaccid paralysis by blocking either neuromuscular transmission, or by targeting muscle sodium channels to block the generation of muscle action potentials. On the other hand, different members of the lightning strike cabal appear to induce excitotoxic shock, with rapid-onset rigid paralysis, by inhibition of sodium channel inactivation and by block of voltage-gated potassium channels. Actions of *Conus* peptides, studied in species other than the natural prey, have revealed cases of unexpected and specific targeting which open possibilities for pharmacological modulation of a variety of processes. Examples include certain μ-conotoxins, nominally considered to be members of the motor cabal, which inhibit particular neuronal sodium channel isoforms more strongly than their canonical target from skeletal muscle. Thus, in a mouse model, μ-conotoxin KIIIA has performed more effectively as an analgesic than lidocaine (Zhang *et al.*, 2007). Conkunitzin-S1, a member of the Kunitz inhibitor family of peptides, blocks certain voltage-gated potassium channels (Bayrhuber *et al.*, 2005) and thereby has the potential to enhance electrical bursting activity. The molecular correlates and physiological consequences of these surprising and striking actions are becoming evident.

Bayrhuber M, Vijayan V, Ferber M, Graf R, Korukottu J, Imperial J, Garrett JE, Olivera BM, Terlau H, Zweckstetter M, Becker S. (2005) *Journal of Biology Chemistry* **280:** 23766-70.

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Calcium, Vc1.1 and α9α10 nicotinic acetylcholine receptors

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Nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels involved in fast synaptic transmission. nAChRs are pentameric complexes formed from a combination of alpha and beta subunits to form heteromeric channels, or alpha subunits alone in the case of homomeric channels. Stoichiometric differences have been conclusively shown to exist with α4β2 nAChR subtypes ((α4)3(β2)2 and (α4)2(β2)3) and that calcium permeability differs between the two receptor populations (Tapia *et al.*, 2007). The α9α10 heteromeric complex is found in inner hair cells, and is potently and selectively inhibited by the conotoxins Vc1.1 and RgIA (Vincler *et al.*, 2006; Halai *et al.*, 2009). Its been shown to exist as one stoichiometric population ((α9)2(α10)3) (Plazas *et al.*, 2005). We have investigated the roles of both stoichiometry of α9α10 receptors and calcium concentration on conotoxin inhibition of ACh-evoked currents heterologously expressed in Xenopus oocytes. We have altered intracellular and extracelluar calcium concentrations, and the ratio of α9 and α10 subunit mRNA to change the relative abundance of the subunits to infer stoichiometry. Our data show that Vc1.1, but not RgIA or atropine, inhibits α9α10 receptors in a biphasic manner under the varying conditions and infer that these receptors exist in at least two stoichiometric forms.

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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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