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LIGANDS TARGETING α10 RECEPTORS TO INHIBIT NEOROPATHIC PAIN

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Abstract

Chronic neuropathic pain is a debilitating problem worldwide that remains inadequately understood and poorly treated. Unfortunately, treatment options for neuropathic pain are limited in number and available options are only partially effective and associated with significant side effects. For example, opioid drugs are often used to treat pain; however, overuse of opioids can lead to drug tolerance over time making them difficult to utilize in chronic pain scenarios. Furthermore, opioids have significant risk factors including dependence, abuse, and overdose. Nonsteroidal anti-inflammatories, or NSAIDS, are a common class of pain medications with fewer side effects and risk factors relative to opioids; however, NSAIDs are only partially effective as analgesics for neuropathic pain. Further investigation is necessary to discover novel analgesic mechanisms and the receptors involved to broaden treatment options.

Nicotinic acetylcholine receptors (nAChRs) are a well-studied family of receptors abundant in the central nervous system that have shown analgesic potential when their activity is modulated by certain ligands. These receptors respond to the endogenous neurotransmitter acetylcholine in the central nervous system and exogenous ligands have been identified that can selectively inhibit or activate them without affecting other types of receptors. However, multiple nAChR subtypes are often expressed in the same tissue or cell type. For example, immune cells that are involved in neuropathic pain are known to express α7 and α9α10 subtypes (Hone & McIntosh, 2018). As a result, pharmacologically targeting individual receptor subtypes in vivo can be very challenging. The lack of subtype-selective ligands has been especially problematic for investigation of α9α10 nAChRs, a subtype that has shown considerable promise as an analgesic target. Ligands that target α9-containing nAChRs specifically have been discovered, however, there are currently no ligands identified for α10; this is largely due to the inability to isolate this receptor subtype in-vitro. Fortunately, novel techniques have been developed to exclusively express α10 homomers in *Xenopus* oocyte models. This research will further investigate potential ligands that target this subtype with high specificity.

When incubated in low concentrations of strychnine, oocytes demonstrated expression of the human α10 receptor ion channel after injection of cRNA. Utilizing the two-electrode voltage clamping technique, current was measured in the oocytes as a function of time. Acetylcholine (ACh) pulses were delivered to the oocyte and changes in the membrane potential were monitored. Various neurotoxins were applied to the bath in an attempt to inhibit ACh-gated responses. Block of the response indicates selective binding between the peptide and receptor. Peptides were initially screened in 101 pools of 10 peptides each. Minimal activity was demonstrated for nearly all peptides and pools at micromolar concentrations; it is possible that peptide interaction within pools may have confounded screening. However, one peptide, Vc1.1[N9W] was determined to consistently inhibit the ACh-induced response, indicating potent inhibition of α10 nAChR. Concentration-response studies indicated that peptide had an IC_{50} of

250 nM. Compared to the native Vc1.1 peptide, this value is 400-fold more potent. The large increase in potency from a single substitution suggests that tryptophan in the ninth position plays a major role in the peptides ability to inhibit the receptor. Initial trials of other native peptides with tryptophan in or near the ninth position so far have not shown block suggesting that the potency enhancing properties of tryptophan may be specific to Vc1.1 The tryptophan residue in the peptide ligand may interact specifically with the receptor or the tryptophan may alter the overall shape of the peptide to bring other residues into more favorable positions to bind to the receptor.

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