1.13 Dicarba modification of α-conotoxin RgIA conferring selectivity towards α9α10 nicotinic acetylcholine receptors

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α-Conotoxins are short disulfide-rich peptides isolated from the venom of predatory marine cone snails and act as nicotinic acetylcholine receptor (nAChR) antagonists. Two α-conotoxins, Vc1.1 and RgIA, have emerged as potential novel therapeutic analgesics. These peptides have two pharmacological targets: the α9α10 nAChR subtype through direct inhibition, and high voltage-activated calcium channel currents indirectly via GABAA receptor activation. However, using these and other conopeptides in research and clinical settings has been hampered by disulfide bridge instability. Disulfide bridges are essential for conopeptide structure and activity, but significantly constrain peptide production, formulation and storage. Dicarba modification of disulfide bridges improved conopeptide stability [1]. In this study, we functionally characterized dicarba-modified α-conotoxin RgIA analogues. Region-selective replacement of the native cysteine framework with a dicarba bridge changed selectivity for its biological targets. [3,12]-Dicarba RgIA selectively inhibited only the α9α10 nAChR subtype with an IC50 of 1.15 μM (95% CI: 0.84–1.55 μM; Hill slope = −1.9) for cis-isomer and 1.47 μM (95% CI: 1.01–2.15 μM; Hill slope = −1.2) for trans-isomer, whereas [2,8]-dicarba RgIA isomers were only active at GABAA receptors and did not inhibit the α9α10 nAChR subtype. A recent study of dicarba Vc1.1 analogues showed similar results, indicating a significant interaction between the α-conotoxin II–IV disulfide bond and GABAA receptors [2]. Interestingly, cis-[3,12]-dicarba RgIA inhibited the α7 nAChR subtype similarly to native RgIA, with an IC50 of 3.73 μM (95% CI: 1.42–9.82 μM; Hill slope = −1.6), whereas the corresponding trans isomer was inactive. Taken together, this study further enhances our understanding about the structure–function relationship of potentially therapeutic α-conotoxins and novel pain targets.

References

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Section 2. In vivo pharmacology and preclinical studies

2.1 Dendritic spine density of prefrontal layer VI neurons is disrupted following chronic in vivo nicotine exposure in development

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In humans, exposure to the drug nicotine in utero substantially raises the risk of developing attention deficits in childhood. This association has been confirmed in rodents, suggesting a teratogenic effect of nicotine on attention circuitry may be responsible. Here, we have treated developing mice chronically with nicotine or vehicle control in order to probe the neurobiological mechanisms underlying the increased vulnerability to attention deficits. We focused on changes in the prefrontal cortex and the neurons in its corticothalamic output layer, in particular, since they have been implicated in top-down control of attentional performance. These layer VI pyramidal neurons would be expected to be vulnerable to developmental nicotine exposure because they have nicotinic acetylcholine receptors that are involved in their excitation and maturation. Our investigation of dendritic spine density in layer VI pyramidal neurons reveals significant changes within nicotine-treated mice as compared to vehicle control mice. These changes likely alter the nature of received excitatory input on layer VI neurons and may contribute to attention deficits following developmental nicotine. To explore further the observed phenomenon, we are currently probing some of the underlying molecular mechanisms involved. Better understanding of the vulnerabilities in attention circuitry during development can provide more general insight into the neurobiological mechanisms of disrupted attention as a result of prenatal exposure to nicotine as well as in other neurobehavioral disorders.

Primary as well as associative regions of the cerebral cortex are thought to be central for aspects of attentional processing. In these regions, top-down projections from layer VI, the deepest cortical layer, exert control over thalamic and thalamocortical circuitry involved in attention. Cholinergic neurotransmission is closely linked to attention, and cholinergic fibers innervate deep layers of both primary and associative cortices. Furthermore, performance of attention tasks is associated with transient increases in acetylcholine in a region-specific manner depending on the nature and demands of the task. However, the electrophysiological effects of acetylcholine, and the specific receptors that mediate its action on cellular targets, are not well understood across cortical regions. Here, we examine the electrophysiological consequences of acetylcholine in layer VI pyramidal neurons of primary and associative cortices.