

NOVEL NONCOMPETITIVE ANTAGONISTS OF NICOTINIC ACETYLCHOLINE RECEPTORS

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Neuronal nicotinic acetylcholine receptors (nAChRs) are involved in a diversity of physiological functions and pathophysiological conditions. Several different nAChR subtypes have been identified. Our laboratory has identified several analogues of methyllycaconitine (MLA) that act as functional inhibitors of bovine adrenal chromaffin nAChRs mediating catecholamine secretion^{1, 2}. In the following studies, we have performed both functional (neurosecretion and calcium influx) and binding experiments to investigate the effects of several new ring E analogues of MLA. We have synthesized a series of analogues that inhibit nicotine-stimulated adrenal neurosecretion (IC₅₀ values ranging from 0.9 to 13 μM). We have subcategorized these analogues based on their effects on KCl-stimulated release and their effects on [³H]epibatidine binding to native nAChRs. These analogues can be divided into three groups. Group I analogues selectively inhibited nAChR-mediated neurosecretion (no effects on 56 mM KCl-stimulated release). These drugs also had little or no effects on agonist binding to native adrenal nAChRs. These data support a noncompetitive interaction of this group of analogues with native nAChRs. Groups II analogues inhibited catecholamine secretion stimulated by both nicotine and KCl (56 mM). Binding studies showed that this group of analogues have no effects on agonist binding to native nAChRs. These data support a site of action of these analogues downstream of membrane depolarization in the stimulus-secretion pathway. The interactions of Group III analogues were much more complex. These analogues inhibited catecholamine secretion stimulated by both nicotine and KCl (as Group II analogues) but increased binding to native nAChRs. Their effects on nicotine-stimulated increase in intracellular calcium concentrations in HEK 293 cells expressing rat α3β4 nAChRs were evaluated as well. Group I analogues showed similar IC₅₀ values to those from neurosecretion, ranging from 0.8 to 5.0 μM. Contrarily, Group II analogues had no more than 40% inhibition, which confirms a nonreceptor-mediated effect of these analogues. The inhibitory effects of Group III analogues were mixed, showing from almost identical to very different IC₅₀ values (higher than 10 μM) to those from neurosecretion. Binding studies were also performed on a stably transfected HEK 293 cell line expressing bovine adrenal α3β4 nAChRs. As predicted, Group I and Group II analogues had little or no effects on agonist binding to recombinant nAChRs. Group III analogues, on the other hand, showed more complex interactions on agonist binding to recombinant nAChRs, ranging from no effect to 35% inhibition. Based on functional and binding data, computational modelling is now being performed to help define the binding site(s) of the analogues.

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2. Bergmeier, S.C., Ismail, K.A., Arason, K.M., McKay, S.B., Bryant, D.L. and McKay, D.B. (2004) *Bioorg. Med. Chem. Lett.* 14, 3739-3742.

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