P016 Muscarinic dualsteric ligands behave as partial or protean agonists depending on the affinity of their orthosteric moiety

Anna De Min[1], Carlo Matera[2], Clelia Dallanoce[2], Ulrike Holzgrabe[3], Marco De Amici[2] and Klaus Mohr[1]
[1]University of Bonn, Bonn, Germany
[2]University of Milan, Milano, Italy
[3]University of Würzburg, Würzburg, Germany

Muscarinic acetylcholine receptors have been extensively studied with the purpose of finding selective ligands for their modulation. In the last years, a new strategy was developed towards this aim, i.e. the synthesis of so-called dualsteric ligands that bind simultaneously to both the orthosteric and the allosteric site of the M_2 receptor.Two dualsteric compounds, Iper-6-naph and Isox-6-naph, and their orthosteric fragments were studied in GTPγS assays and [^{3}H]NMS binding studies, performed with membranes of CHO cells expressing the human M_2 receptor. The experiments were carried out in Tris buffer supplemented with either 2 mM or 200 mM NaCl, in order to have the receptor in its spontaneously active or inactive conformations, respectively. In both buffers, Iper-6-naph was a partial agonist and it almost completely abolished [^{3}H]NMS binding. In contrast, Isox-6-naph revealed a protean nature, behaving either as inverse or partial agonist, respectively, in 2 mM and 200 mM NaCl, and it promoted [^{3}H]NMS binding. Since the two hybrid compounds share the same allosteric moiety, these discrepancies have to be attributed to pharmacological differences of the orthosteric moieties, whose potencies and affinities were indeed reported to be significantly divergent. In conclusion, this study suggests that slight structural modifications in the orthosteric building block of dualsteric ligands may cause a reversal of ligand efficacy and, most importantly, it provides a deeper insight into the molecular determinants of protean agonism at the M_2 receptor.