Engineered α4β2 nicotinic acetylcholine receptors as models for measuring agonist binding and effect at the orthosteric low-affinity α4-α4 interface

The nicotinic acetylcholine receptor alpha 4 beta 2 is important for normal mammalian brain function and is known to express in two different stoichiometries, (alpha 4)(2)(beta 2)(3) and (alpha 4)(3)(beta 2)(2). While these are similar in many aspects, the (alpha 4)(3)(beta 2)(2) stoichiometry differs by harboring a third orthosteric acetylcholine binding site located at the alpha 4-alpha 4 interface. Interestingly, the third binding site has, so far, only been documented using electrophysiological assays, actual binding affinities of nicotinic receptor ligands to this site are not known. The present study was therefore aimed at determining binding affinities of nicotinic ligands to the alpha 4-alpha 4 interface. Given that epibatidine shows large functional potency differences at alpha 4-beta 2 vs. alpha 4-alpha 4 interfaces, biphasic binding properties would be expected at (alpha 4)(3)(beta 2)(2) receptors. However, standard saturation binding experiments with [H-3]epibatidine did not reveal biphasic binding under the conditions utilized. Therefore, an engineered beta 2 construct (beta 2(HQT)), which converts the beta(-) face to resemble that of an alpha 4(-) face, was utilized to create (alpha 4)(3)(beta 2(HQT))(2) receptors harboring three alpha 4-alpha 4 interfaces. With this receptor, low affinity binding of epibatidine with a K-d of similar to 5 nM was observed in sharp contrast to a K-d value of similar to 10 pM observed for wild-type receptors. A strong correlation between binding affinities at the (alpha 4)(3)(beta 2(HQT))(2) receptor and functional potencies at the wild-type receptor of a range of nicotinic ligands highlighted the validity of using the mutational approach. Finally, large differences in activities at alpha 4-beta 2 vs. alpha 4-alpha 4 interfaces were observed for structurally related agonists underscoring the need for establishing all binding parameters of compounds at alpha 4 beta 2 receptors. Crown Copyright (C) 2015 Published by Elsevier Ltd. All rights reserved.