

PHARMACOLOGICAL CHARACTERIZATION OF SINGLE NUCLEOTIDE POLYMORPHISMS FOR HUMAN CB₂ RECEPTOR

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The cannabinoid system plays a crucial role in the mechanism of nociception. In particular, CB₂ receptor is considered a new target for neuropathic pain therapies. Since the CB₂ receptor is expressed in the periphery and only in some districts of the CNS, CB₂ selective agonists should elicit analgesic effects without displaying psychotropic side effects typical of the CB₁ ligands.

Single nucleotide polymorphism (SNP) which cause amino acid changes in receptor sequence could alter its biochemical properties and then influence the efficacy of ligands targeted to this receptor. Thus, in the drug discovery process when searching for selective CB₂R agonists, it is important to test if the compounds of interest show a different pharmacological profile on the SNPs compared to the wild type receptor.

For human CB₂ gene have been identified three SNPs which lead to changes in the amino acid sequence of the receptor and that are present in more than 10% of the population. This SNPs are Arginine63Glutamine and Arginine66Glutamine, in the first intracellular loop and Hystidine316Tyrosine, in the C-terminal tail.

To assess the effect of SNP on CB₂ receptor ligands, we have generated several CHO cell lines stably expressing each of the most frequent SNPs. We have pharmacologically characterized all clones, using as CB agonist WIN55212-2 and identified a representative clone for each SNP. On these representative clones we have completed pharmacological characterization using reference CB agonists (CP55940, JWH133, GW842166X, AM1241), by measuring EC₅₀ and E_{max} using a functional assay based on the reduction of forskolin-induced release of cAMP and K_i using a binding assay based on [³H]-WIN55212-2 displacement.

The results of this characterization will be presented and discussed comparing the pharmacological profile of each reference compound in the wild type and each SNP receptor.