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Mutation of a Transposed Amino Acid Triplet Repeat Enhances Coupling of m1 Muscarinic Receptor to Activation of Phospholipase C

Seok-Yong Lee° and Tai-Soon Cho*

Department of Pharmacology, Catholic University Medical College
*Department of Pharmacology, College of Pharmacy, Sung Kyun Kwan University**

The C-terminus ends of the second putative transmembrane domains of both m1 and m2 muscarinic receptors contain a triplet of amino acid residues consisting of leucine (L), tyrosine (Y) and threonine (T). This triplet is repeated as LYT-LYT in m2 receptors at the interface between the second transmembrane domain and the first extracellular loop. Interestingly, however, it is repeated in a transposed fashion (LYT-TYL) in the sequence of m1 receptors. In this work we employed site-directed mutagenesis to investigate the possible significance of this unique sequence diversity for determining the distinct differential drug-receptor interaction and cellular function at m1 muscarinic receptor. Mutation of the LYTTYL sequence of m1 receptors to the corresponding m2 receptor LYTLYT sequence, however, did not result in a significant change in the binding affinity of the agonist carbachol or in the affinity of the majority of a series of receptor antagonists which are able to discriminate between wild-type m1 and m2 receptors. Surprisingly, the LYTLYT m1 receptor mutant demonstrated markedly enhanced coupling to activation of phospholipase C without a change in its coupling to increased cyclic AMP formation. There was also an enhanced receptor sensitivity in transducing elevation of intracellular Ca^{2+}. These changes were not due to alterations in the rate of receptor desensitization or sequestration. On the other hand, the reverse LYTLYT→LYTTYL mutation in the m2 receptor did not alter its coupling to inhibition of adenylate cyclase, but slightly enhanced its coupling to stimulation of PI hydrolysis. Our data suggest that the LYTTYL/LYTLYT sequence difference between m1 and m2 muscarinic receptors is not involved in determining receptor pharmacology. On the other hand, while these differences might play a role in the modulation of muscarinic receptor coupling to PI hydrolysis, they are not important for specifying coupling of various subtypes of muscarinic receptors to different cellular signaling pathways.