ABSTRACT

Atypical agonists of muscarinic receptors bind to individual receptor subtypes with comparable affinity but activate them selectively to a certain extent. Molecular mechanism underlying this "functional selectivity" is not known and its elucidation may contribute to development of new atypical functionally selective agonists suitable for therapeutic use.

Functional selectivity of atypical muscarinic agonists may be caused by a distinct molecular mechanism(s) of how these compounds activate the receptor. Agonist-specific conformations induced by structurally complex atypical agonists may lead to utilization of a parallel activation mechanism that is different than the activation mechanism induced by non-selective classical agonists. In order to examine this possibility we investigated whether the M_1 receptor preferring atypical agonists xanomeline and N-desmethylclozapine, and the classical orthosteric agonists carbachol and oxotremorine, activate the M_1 receptor through a common cascade of transmission switches.

To this end we mutated key amino acids of the M_1 receptor that are essential for ligand binding to the orthosteric binding site ($D105^{3.32}$, $D99^{3.26}$), receptor activation (transmission switch, $D71^{2.50}$), or interaction with G-protein (ionic lock switch, $R123^{3.50}$ $D122^{3.49}$). We compared effects of these mutations on binding characteristics and functional responses of atypical and classical agonists. Furthermore we analyzed effects of these mutations on persistent activation of the M_1 receptor by wash-resistantly bound xanomeline.

Point mutation of $D105^{3.32}$ and $D99^{3.26}$ decreased affinity of all tested agonists and caused a decrease in potency of receptor activation in functional assays. Mutation of $D105^{3.32}$ in the orthosteric binding site decreased the potency of the atypical agonist xanomeline more than the potency of the classical orthosteric agonists carbachol and oxotremorine. Point mutation of residue $D71^{2.50}$, involved in receptor activation, or $R123^{3.50}$, involved in coupling to G-proteins, completely abolished functional responses to both classical and atypical agonists.

Our data show that classical as well as atypical agonists activate the M_1 receptor by the same molecular mechanism that involves the transmission switch $D71^{2.50}$ and the ionic lock switch ($R123^{3.50}$, $D122^{3.49}$). These results further point to the key role of $D105^{3.32}$ in the orthosteric binding site in receptor activation by atypical agonists and persistent activation of the M_1 receptor by wash-resistantly bound xanomeline. The principal difference among tested agonists is in the way they interact with $D105^{3.32}$. In addition, our data demonstrate an important role of the vestibule of the orthosteric binding site for atypical agonists binding.