ABSTRACT

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Muscarinic acetylcholine receptors (mAChRs), members of the superfamily of G-protein coupled receptors (GPCRs), regulate vital physiological processes and are important targets in drug research. Five different subtypes (M_1 – M_5) have been identified. M_1 mAChR is mainly distributed in the central nervous system and is linked to pathophysiology of neurodegenerative diseases. In recent years, fluorescent methods have been frequently used in studies of ligand binding to receptors. The fluorescence anisotropy (FA) is a homogenous assay to characterize ligand binding to receptors.

In this work, we have evaluated the FA method with fluorescent ligand MK342 binding to M_1 mAChRs expressed on budded baculovirus (BBV) particles. The fluorescence ligand was binding with the high affinity (4,4 nM) to M_1 receptor in constructed BBV preparation. The apparent binding affinities (pK_i) of eleven classical and three bitopic muscarinic ligands were screened and compared to previously published literature. In most cases, the affinity values of competitors determined in FA assay were lower than previously published but the linear correlation analysis with $R^2 = 0,95$ shows the FA method can be used to characterise ligand binding. The on-line monitoring of competitive binding with bitopic ligands indicates a more complex mode of binding therefore it appears that MK342 can be also used in studies of allosteric modulation.