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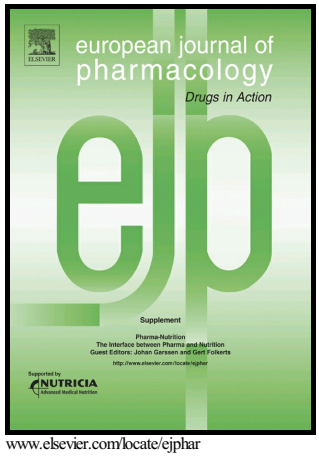

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Implementation of fluorescence anisotropy-based assay for the characterization of ligand binding to dopamine D_1 receptors

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Abstract

Dopamine receptors, which belong to the family of G protein-coupled receptors, are very substantial regulators in the brain and therefore important targets in drug discovery. Radioligand binding assay has been the method of choice for screening novel dopaminergic drugs for decades. We demonstrate that fluorescent ligand BodipyFL-SKF83566 binding to dopamine D_1 receptors expressed in baculovirus particles can be characterized with fluorescent anisotropy (FA) based assay and that this is a valuable alternative to the radioligand binding assay. High binding affinity (K_D = 5.2 nM) and fast association and dissociation kinetics (half-lives 40 s and 70 s, respectively) make BodipyFL-SKF83566 a suitable fluorescent ligand for this type of experiments. Good correlation between pK_i values of 11 different dopaminergic ligands determined in competition binding experiments with radioligand [^{3}H]SCH23390 or BodipyFL-SKF83566 (R^2 = 0.96, slope not significantly different from unity) further validates the FA assay. In addition to competitor's affinity, the method also enables to quantify the apparent pIC_{50} values in time and hence kinetic properties of an unlabeled ligand can be estimated from the same competition binding