Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol19Issue1abst012P.pdf

A dopamine D1 receptor binding assay using bioluminescence resonance energy transfer (BRET) with Nanoluciferase

J. Appelhans[1], A. J. Brown[2], P. J. McCormick[3]. [1]Queen Mary University of London, London, United Kingdom, [2]GlaxoSmithKline, Stevenage, United Kingdom, [3]William Harvey Research Institute, London, United Kingdom.

Introduction: Pharmaceutical companies test candidate therapeutic agents *in vitro* for activity at receptors involved in adverse effects early in the hit and lead identification process. Binding assays are an important part of this process but traditional radioligand methods can be restrictive for practical reasons. Here we describe a bioluminescence resonance energy transfer (BRET) competition binding assay between a fluorescently tagged D1R ligand (CA200773, HelloBio) and untagged ligands at the Dopamine D1 receptor (D1R), a receptor with a propensity to cause adverse neuropsychological effects. Our aim was to develop a robust screening assay at the D1R using NanoBRET.

Method: Nanoluciferase (Nluc) was cloned onto the D1R N-terminus with a GSSG linker. Functionality of the Nluc-D1R fusion was demonstrated by stimulation of cAMP accumulation with D1R agonists in transfected HEK293 cells. Antagonism of CA200773 was confirmed by its ability to inhibit SKF81297-induced cAMP accumulation in wild-type D1R-transfected HEK293 cells. Specificity for the D1R was shown by fluorescent microscopy of CA200773-treated D1R-transfected and untransfected HEK293 cells. A BRET saturation binding experiment was conducted on Nluc-D1R-transfected HEK293 cells using increasing concentrations of CA200773. An unlabelled competing ligand (SKF83566) was used to assess non-specific binding. To determine if this competition of BRET interaction could constitute a screen for ligands binding at the D1R, we tested a panel of 28 ligands previously shown to inhibit agonist-evoked cAMP accumulation in D1R-expressing cells. Ligands were tested over a range of concentrations with Nluc-D1R-transfected HEK293 cells in the presence of CA200773. Z-prime, a measure of statistical effect size, was derived by comparing vehicle-treated and SKF83566-treated cells on each 384-well microtitre plate.

Results: cAMP accumulation levels at Nluc-D1R were comparable to the wild-type. Calculated IC_{50} of CA200773 was similar to that specified by the manufacturer and CA200773 was shown by fluorescent microscopy to bind specifically at HEK293 cells expressing D1R but not at parent HEK293 cells. IC_{50} values for BRET inhibition were consistent across experiment occasions (R^2= 0.94). Unexpectedly, the 28 screened ligands fell into two groups. For some, IC_{50} values correlated well between inhibition of BRET compared to inhibition of cAMP accumulation (R^2=0.65). For others, no BRET inhibition was detected, potentially due to susceptibility of the cAMP assay to false positives. Over two separate assay occasions, Z' for BRET inhibition was consistently >0.5 (Z'= 0.67±0.08; mean±SD) indicating a large statistical effect.

Conclusion: Inhibition of BRET is a valid approach to measure ligand interaction at the dopamine D1 receptor.