Fluorescence and bioluminescence approaches to study ligand binding to GPCRs and RTKs

Stephen J. Hill

University of Nottingham & President, British Pharmacological Society, UK

Previous work in our lab, using fluorescent agonists and antagonists, has provided novel insights into the allosteric regulation of adenosine A3 (A3AR), A1 (A1AR) receptors and beta1-adrenoceptors by allosteric ligands and receptor dimerization in single living cells (1,2). Fluorescence correlation spectroscopy (FCS) has also been used to investigate ligand binding to A1AR and A3AR in small $0.2 \mu\text{m}^2$ microdomains of single living cells (3,4). FCS studies with a fluorescent A3-agonist have enabled high affinity labeling of the active conformation (R^*) of the receptor (3,4) whilst a fluorescent adenosine A3-antagonist (CA200645) has been used to study the binding characteristics of antagonist-occupied receptor conformations (R) in membrane microdomains of single cells (3). Investigation of the dissociation kinetics of CA200645 by FCS has also provided further support for allosteric regulation of this receptor by homodimerization (3).

More recently, we have developed novel ligand binding assays for both G protein-coupled receptors (GPCRs) and receptor tyrosine kinases (particularly VEGFR2) using cell surface receptors tagged with a novel N terminal luciferase (NanoLuc; Promega) and bioluminescence resonance energy transfer (BRET) to a fluorescent ligand (5-7). My presentation will summarize some of our recent developments with fluorescent ligands and NanoBRET technologies.

- (1) May LT et al (2011), FASEB J 25:3465-76
- (2) Gherbi K et al (2015) FASEB J 29: 2859-71
- (3) Corriden R et al (2014) FASEB J 28: 4211-4222
- (4) Briddon SJ et al (2018) Trends in Pharmacol Sci in press.
- (5) Stoddart LA et al (2015) Nature Methods 12: 661-663.
- (6) Kilpatrick LE et al. (2017) Biochem Pharmacol 136: 62-75.
- (7) Stoddart LA et al (2018) Trends Pharmacol Sci in press