BJP BRITISH JOURNAL OF PHARMACOLOGY

BPS BRITISH PHARMACOLOGICAL SOCIETY

British Journal of Pharmacology (2010), 159, 735–737

0007-1188/10
www.brjpharmacol.org

THEMED SECTION: IMAGING IN PHARMACOLOGY

EDITORIAL

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This themed section on Imaging in Pharmacology comprising reviews and original articles arose from two symposia held at the Summer Meeting of the British Pharmacological Society in Edinburgh 8–10 July 2009 on Developments in Receptor Imaging and Imaging and Targeting Inflammation in Stroke and Atherosclerosis. The reports cover a broad spectrum of pharmacological studies, from whole animal imaging to gene expression and emphasize the importance of imaging techniques in pharmacology. The development of each new imaging methodology brings pharmacology closer to the ambitious goal of being able to image (simultaneously) each component part of the G-protein-coupled receptor signalling process.
British Journal of Pharmacology (2010) **159,** 735–737; doi:10.1111/j.1476-5381.2010.00685.x

This article is part of a themed section on Imaging in Pharmacology. See the reference list for all papers appearing in this section.

Keywords: G-protein coupled receptors, fluorescence imaging, confocal microscopy, positron emission tomography, gene therapy

A picture is said to be worth a thousand words but its value increases substantially for pharmacologists with the ability to not only visualize biomolecules but extract quantitative information about their interactions using image analysis, ideally in real time and non-invasively. As a result, imaging has become an important tool in virtually every branch of the life science. Developments in imaging receptors both *in vitro* and *in vivo* are rapidly progressing with enhancements to both hardware and software which in turn leads to important new methodologies and applications. This themed section on Imaging in Pharmacology comprising two invited reviews and five original articles arose from two symposia held at the Summer Meeting of the British Pharmacological Society in Edinburgh 8–10 July 2009 on Developments in Receptor Imaging organized by Craig Daly (Glasgow, UK) and Imaging and Targeting Inflammation in Stroke and Atherosclerosis organized by Pasquale Maffia, Paul Coats, Hilary Carswell (Strathclyde Institute of Pharmacy & Biomedical Sciences, University of Strathclyde, Glasgow, UK).

G-protein-coupled receptors (GPCRs) transduce an eclectic diversity of signals ranging from single photons of light through to peptides and small proteins. GPCRs, particularly from Family A or Class 1 represent targets for nearly half of currently available medicines and continue to be a major focus for the development of novel imaging agents, particularly fluorescently labelled biomolecules (Daly and McGrath, 2003; Briddon and Hill, 2007).

Rose (2010) provides a succinct overview of applying the emerging technique of bimolecular fluorescence complementation to GPCR signalling particularly measuring interactions with G-proteins and β-arrestin. This technique involves the division of a protein reporter into two non-functioning fragments, each fused to the separate partners under investigation. The association of the target proteins then drives recombination of the reporter fragments, yielding measurable functional activity usually fluorescence excitation/emission characteristics of the parent protein. The technique has the potential to answer key questions as to how GPCRs select downstream signalling partners and how these are compartmentalized. Perhaps the most exciting prospect is studying GPCR homodimerization and heterodimerization.

In humans, gonadotrophin-releasing hormone (GnRH) mediates its actions by GPCRs that uniquely have evolved in mammals to lack C-terminal tails that are usually necessary in other receptors for agonist-induced phosphorylation and arrestin binding, desensitization, internalization and signalling (McArdle *et al.*, 2002). Finch *et al.* (2010) describe an automated fluorescence strategy to demonstrate, as a consequence of this structural modification, GnRH receptors are poorly expressed on the cell surface, most likely reflecting inefficient exit from the endoplasmic reticulum. Significantly, pharmacological intervention using GnRHR antagonists can

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Received 5 January 2010; revised 6 January 2010; accepted 7 January 2010

increase cell surface GnRH receptor expression. As GnRH mediates control of reproduction by stimulating gonadotrophin secretion from the pituitary, these imaging techniques provide a mechanism for testing the efficacy of pharmacological chaperones to increase cell surface receptor expression to provide the rationale for the potential treatment of infertility.

Radiolabelled ligands have been widely used to characterize GPCRs for four decades but with the exception of autoradiography combined with electron microscopy, visualization of receptors is limited to tissues or groups of cells. The great advance in the use of fluorescent labelled ligands is that receptor pharmacology can be visualized and quantified in a single cell. However, unlike many isotopes used to radiolabel ligands, the size of most fluorophores in relation to the ligand may result in alterations to the pharmacology of the parent molecule. Using the adenosine A_1 as a GPCR target, Baker *et al.* (2010) have synthesized an extensive range of fluorescent ligands which have been pharmacologically characterized by inhibition of binding of [^{3}H]DPCPX to expressed receptors. They show that binding is influenced not only by the type of fluorophore but also the length of the associated linker. Intriguingly, in living cells, the physicochemical properties of the fluorophore/linker pairing determine where in the environment of the target receptor the fluorophore is placed and this property may ultimately determine the utlility of a particular compound as a fluorescent probe.

Daly *et al.* (2010) have exploited the increase in spatial precision that can be achieved by using confocal imaging with fluorescent labelled ligands to visualize receptors binding four dissimilar fluorescent ligands (α_1-adrenoceptors, β-adrenoceptors, angiotensin II and cannabinoid receptors) in arteries. Within each cell type within the vessel wall including smooth muscle and endothelial cells, each GPCR examined had a distinctive heterogeneous distribution with limited co-localization with other receptors. Such studies using native tissues are important as many interactions between receptors such as heterodimerization assume that both receptors are expressed in the same cell. While heterodimers may be identified in cell lines expressing comparatively high densities of receptors, it is important to determine whether cells from native tissues are also capable. This approach has recently been exploited in vascular smooth muscle cells (Methven *et al.*, 2009a,b).

Fluorescence imaging is not limited to visualizing receptors but has the potential to quantify the uptake of transmitters. The extracellular concentration of noradrenaline depends on its rate of release by exocytosis balanced by its rate of uptake, which in the peripheral nervous system is the norepinephrine transporter (NET). Parker *et al.* (2010) used confocal microscopy to visualize the accumulation of NET substrate in the mature sympathetic nerve terminals of the mouse isolated vas deferens. They showed fluorescence accumulated linearly in nerve terminals, which could be blocked by inhibitors of NET inhibition (desipramine) and reversed by amphetamine. This technique has the potential to study the physiology of NET regulation and the action of NET inhibitors on mature sympathetic terminals in a single nerve terminal varicosity.

A major challenge in imaging is to translate results from florescent probes *in vitro* to *in vivo*. Ortolano *et al.* (2010) describe their approach towards imaging fluorescent labelled lymphocytes injected into mice 24 h before undergoing permanent left middle cerebral artery occlusion (MCO), an animal model of stroke. Although brains were subsequently cut into section for imaging, recruitment of labelled lymphocytes in the cerebrovasculature and in the cortex, downstream of occluded middle cerebral artery in adoptive transfer mice, was observed at 24 and 48 h MCO using multiphoton laser scanning microscopy. These results suggest direct observations of the dynamic T-cell behaviour can be made in living brain tissue in real time using this technique as well as *ex vivo* live imaging of immune response after experimental stroke.

Positron emission tomography (PET) is a functional imaging technique that is used to study biological processes *in vivo*. It is the most sensitive technique available to image and quantify receptor distributions *in vivo,* particularly with the development of tomographs dedicated to small animals such as rodents for preclinical imaging. It has been used extensively to study major neurotransmitter systems and data obtained in a PET scan can provide information regarding tissue physiology or pathophysiology, as well as pharmacokinetic and pharmacodynamic information (see e.g. Johnström *et al.*, 2005a,b). PET has increasingly been recognized as a very powerful tool to accelerate development and assessment of existing and novel drugs. Johnström *et al.* (2010) report for the first time the use of PET to dynamically image *in vivo* within target organs the tissue specific conversion of the biologically inactive precursor [^{18}F]-big endothelin-1 to the mature peptide [^{18}F]-endothelin-1 as binding to endothelin receptors. A major site for conversion was within the vasculature of the lung and liver, whereas uptake in kidney was more complex reflecting excretion of [^{18}F]-Big ET-1 without conversion to ET-1.

Finally, in the new era of regenerative medicine, Baril *et al.* (2010) review strategies for non-invasive imaging of gene therapy, to allow the precise spatiotemporal measurement of gene expression particularly in longitudinal studies involving gene transfer vectors. For the imaging technique to be effective, it should provide precise information about the location, magnitude and persistence of a specific transgene over time. The authors focus on the sodium iodide symporter (NIS), describing the biological function of the system to transport molecules across a phospholipid membrane using an electrochemical gradient of sodium ions (Na+) as carrier and an adenosine triphosphate-driven pump. The imaging potential of NIS to monitor gene expression in the live subject using nuclear imaging technology is considered including trafficking of stem cells.

Many pharmacological studies result in the generation of an image of some sort such as a blot, gel or photomicrograph. However, as alluded to above, a number is worth a thousand pictures and it is important to extract as much quantitative information as possible from our images. With the developments of live cell imaging and ultra fast scanning, it is now possible to visualize biological events in real time. The reports published in this 'imaging' themed section cover a broad spectrum of pharmacological studies, from whole animal imaging to gene expression. These reports emphasize the

importance of imaging techniques in pharmacology. Furthermore, the development of each new fluorescent probe and imaging methodology brings us ever closer to the goal of being able to image (simultaneously) each component part of the GPCR signalling process.

British Journal of Pharmacology welcomes manuscripts employing imaging technologies to study pharmacological processes and is developing ways to support authors, such as online supplementary data that can be in movie or 3D display formats. Note that colour in figures is completely free of cost to the author.

References

Baker JG, Middleton R, Adams L, May LT, Briddon SJ, Kellam B *et al*. (2010). Influence of fluorophore and linker composition on the pharmacology of fluorescent adenosine A1 receptor ligands. *Br J Pharmacol* **159**: 772–786.

Baril P, Martin-Duque P, Vassaux G (2010). Visualization of gene expression in the live subject using the Na/I symporter as a reporter gene: applications in biotherapy. *Br J Pharmacol* **159**: 761–771.

Briddon SJ, Hill SJ (2007). Pharmacology under the microscope: the use of fluorescence correlation spectroscopy to determine the properties of ligand-receptor complexes. *Trends Pharmacol Sci* **28**: 637–645.

Daly CJ, McGrath JC (2003). Fluorescent ligands, antibodies, and proteins for the study of receptors. *Pharmacol Ther* **100**: 101–118.

Daly CJ, Ross RA, Whyte J, Henstridge CM, Irving AJ, McGrath JC (2010). Fluorescent ligand binding reveals heterogeneous distribution of adrenoceptors and 'cannabinoid-like' receptors in small arteries. *Br J Pharmacol* **159**: 787–796.

Finch AR, Sedgley KR, Armstrong SP, Caunt CJ, McArdle CA (2010). Trafficking and signalling of gonadotrophin-releasing hormone receptors: an automated imaging approach. *Br J Pharmacol* **159**: 751–760.

Johnström P, Fryer TD, Richards HK, Barret O, Davenport AP (2005b). Dynamic in vivo imaging of receptors in small animals using positron emission tomography. *Methods Mol Biol* **306**: 217–232.

Johnström P, Fryer TD, Richards HK, Harris NG, Barret O, Clark JC *et al*. (2005a). Positron emission tomography using ^{18}F-labelled endothelin-1 reveals prevention of binding to cardiac receptors owing to tissue-specific clearance by ET_B receptors in vivo. *Br J Pharmacol* **144**: 115–122.

Johnström P, Fryer TD, Richards HK, Maguire JJ, Clark JC, Pickard JD *et al*. (2010). Positron emission tomography of [^{18}F]-big endothelin-1 reveals renal excretion but tissue specific conversion to [^{18}F]- endothelin-1 in lung and liver. *Br J Pharmacol* **159**: 812–819.

McArdle CA, Franklin J, Green L, Hislop JN (2002). Signalling, cycling and desensitisation of gonadotrophin-releasing hormone receptors. *J Endocrinol* **173**: 1–11.

Methven L, McBride M, Wallace GA, McGrath JC (2009b). The $\alpha_{1B/d}$-adrenoceptor knockout mouse permits isolation of the vascular α_{1A}-adrenoceptor and elucidates its relationship to the other subtypes. *Br J Pharmacol* **158**: 209–224.

Methven L, Simpson PC, McGrath JC (2009a). $\alpha_{1A/B}$-Knockout mice explain the native α_{1D}-adrenoceptor's role in vasoconstriction and show that its location is independent of the other α_{1D}-subtypes. *Br J Pharmacol* **158**: 1663–1675.

Ortolano F, Maffia P, Dever G, Rodolico G, Millington OR, De Simoni MG *et al*. (2010). Advances in imaging of new targets for pharmacological intervention in stroke: real-time tracking of T-cells in the ischaemic brain. *Br J Pharmacol* **159**: 808–811.

Parker LK, Shanks JA, Kennard JAG, Brain KL (2010). Dynamic monitoring of NET activity in mature murine sympathetic terminals using a fluorescent substrate. *Br J Pharmacol* **159**: 797–807.

Rose RH, Briddon SJ, Holliday N (2010). Bimolecular fluorescence complementation: lighting up seven transmembrane domain receptor signalling networks. *Br J Pharmacol* **159**: 738–750.