British Journal of Pharmacology (2003) 139, 187–189
www.nature.com/bjp

COMMENTARY

Do fluorescent drugs show you more than you wanted to know?

*,[1]J.C. McGrath & [1]C.J. Daly

[1]Autonomic Physiology Unit, Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow G12 8QQ, Scotland

British Journal of Pharmacology (2003) **139,** 187–189. doi:10.1038/sj.bjp.0705288

Keywords: β_2-Adrenoceptors; fluorescent ligands; CGP 12177; partial agonism; GPCR; plasmalemmal receptors; intracellular receptors; BODIPY TMR-CGP; ligand binding

An article in this issue of *BJP* documents the cellular pharmacology and cellular distribution of a fluorescent ligand for β_2-adrenoceptors, BODIPY TMR-CGP 12177. This commentary discusses issues surrounding the use of fluorescent ligands including the discipline necessary for their successful exploitation and the additional properties of drugs and receptors that they bring into focus.

Fluorescence-based assays have become ubiquitous in biomedical science. A few years ago there seemed bright prospects for using fluorescently tagged drug molecules to gain insight into pharmacological mechanisms (McGrath *et al*., 1996). The ability to visualise ligand – receptor interactions with spatial and temporal resolution should enable us to bring to physical reality phenomena involved with drug – receptor interactions that remain hypothetical.

However, there was no subsequent explosion of knowledge or emergence of new fluorescent ligands and the field has been limited to a few ligands for α_1-adrenoceptors (Daly *et al*., 1998; McGrath *et al*., 1999a, b; MacKenzie *et al*., 2000), opioid (Arttamangkul *et al*., 2000; Madsen *et al*., 2000), neuropeptides (Jenkinson *et al*., 1999; Fabry *et al*., 2000; Buku *et al*., 2001), angiotensins (Von Bohlen und Halbach & Albrecht, 2000), bradykinin (Howl, 1999) and vasopressin (Tran *et al*., 1999). In general, fluorescently labelled drugs have been used in a very passive way, literally as labels rather than exploiting their characteristics as agonists or antagonists. This may reflect scepticism by pharmacologists about the pharmacological properties of compounds that have been radically changed by the addition of large fluorescent moieties. Alternatively, the lack of an accepted standardised quantitative method for their analysis may have reduced their appeal.

These issues are raised in an article in this issue of *BJP* by Jillian G. Baker, Ian P. Hall, and Stephen J. Hill entitled 'Pharmacology and direct visualisation of BODIPY TMR-CGP: a long acting fluorescent β_2-adrenoceptor agonist'. In this they first document the pharmacology of the compound in conventional terms at β_2-adrenoceptors (radioligand binding *versus* ^{3}H-CGP12177) and functional studies using a cyclic AMP response element-mediated gene transcription method and go on to use this pharmacological data in the construction of experiments designed to show the localisation of recombinant β_2-adrenoceptors that are accessible to the drug in CHO-K1 cells.

*Author for correspondence; E-mail: j.c.mcgrath@bio.gla.ac.uk

The same authors had shown that the parent drug, CGP 12177, in the same cell system, is a partial agonist at β_2-adrenoceptors (Baker *et al*., 2002). CGP 12177 has been used as an experimental drug for many years. A tritiated version has been used as a high affinity ligand at β_1- and β_2-adrenoceptors and it is also considered to be a low-affinity partial agonist at β_3-adrenoceptors (refs from Baker *et al*., 2002).

CGP 12177 was one of the first ever examples of a fluorescently labelled drug to be made and deployed pharmacologically (Heithier *et al*., 1994). A comparison of FITC and BODIPY conjugates favoured the latter. These compounds never became available commercially and to the best of our knowledge the BODIPY (green) form was employed in only one further published study (Arribas *et al*., 1997). A commercial compound with a different spacer (part of molecule linking parent compound and fluorescent moiety) subsequently became available (Thorlin *et al*., 2000). The compound employed by Baker *et al*., has a similar spacer to the latter compound, but a different fluorescent moiety, namely 'Bodipy-TMR'. This illustrates one of the drawbacks to a narrow field, that is, that different but similar-sounding drugs might be used, and emphasises the importance of testing the pharmacology of fluorescent compounds in the system in which they are employed, as Baker *et al*. (2003) have done.

In the course of their study it transpired that the fluorescent compound, BODIPY TMR-CGP, was also a partial agonist at β_2-adrenoceptors, but the authors emphasise that it had an exceedingly long persistence on the receptor, which they showed by radioligand binding and functional studies as well as by being able to visualise it long after 'washout'. This is an excellent example of the type of property of a drug that comes to light when it can be visualised. It triggers two lines of thought.

First, how interesting it is that this drug has such a slow off-rate. What does that do for its kinetics, does this complicate quantitative analysis of its pharmacology and does it affect its distribution and therefore confound the point of having a visualisable drug? Is this property because of some allosteric effect of the fluorescent moiety (Coleman *et al*., 1996) or to a change in the charge or conformation of the molecule where it interacts with the receptor? We can easily check the pharmacology of the fluorescent-derivative, but how is the chemistry changed? Might it become more or less lipophilic when the fluorescent group is attached?

Secondly, how often does anyone wash out an antagonist or partial agonist? How many other drugs have a very long persistence that is not noticed? This latter point illustrates the

generally thought provoking nature of this class of drugs. They give us additional information that we do not normally have for nonfluorescent drugs. Sometimes this does not fit our preconceptions. With fluorescent ligands we are seeing specific and nonspecific (i.e. total) binding. Sometimes this is too much information.

This may finally explain why the use of fluorescent drugs in pharmacology has not taken off. As persistent pioneers in this arena we have been frequently told by colleagues that they have tried fluorescent drugs, but that they 'did not work'. This usually means either that they saw fluorescence where they did not expect to find it, for example, on the 'wrong' cell type or inside the cells instead of on the surface, or that the fluorescence was 'nonspecific' because it persisted in the presence of high concentrations of antagonists for that class of receptor. For example, in blood vessels 'vasoactive' receptors are anticipated to be present on vascular smooth muscle, but they turn up on other cell types including nerves, endothelium and adventitia (Brahmadevara *et al*., 2002; McGrath *et al*., 2002).

In the Baker *et al.* (2003) paper, there is only one cell type and the relevant receptors are programmed experimentally. However, the danger of binding in the wrong or unexpected place had to be addressed. Recombinant GFP-receptor fusion proteins showed that receptors are indeed found inside the cells and high concentrations of the BODIPY TMR-CGP (300 nM but not 100 nM) bind inside the cells. The matter was resolved by showing that the β_2-adrenoceptor antagonist ICI 118551 prevented binding to the cell rim, but not inside, suggesting that the latter was nonspecific. However, what if ICI 118551 cannot enter the cell, whereas BODIPY TMR-CGP can? Are we now asking unreasonable questions that would not be demanded in the nonfluorescent world? Who considers that an antagonist drug that they are using might enter cells and bind there with consequences for its kinetics? To return to the colleagues with the 'failed' experiments: who would try out a new compound at less than 300 nM?

The prospects for fluorescent drugs should remain good providing that they are analysed in depth as in Baker *et al*. (2003). The possibilities for visualising both the drugs and recombinantly labelled receptors (Milligan, 1999) extend to interactions that can be analysed by fluorescence resonance energy transfer (FRET) based methods (e.g. BRET, PbFRET, FLIM, etc.), but will be convincing only if the drug is well-characterised. Similarly trafficking of receptors with their cargo of fluorescent ligand offers attractive scenarios (Vandenbulcke *et al*., 2000), particularly if fluorescent agonists and antagonists can both be used.

The wider use of fluorescence technologies in combination offers attractive options. A recent paper in *BJP* by Jackson & Cunnane (2002) (see also McGrath, 2002) shows that activity in nerves as well as smooth muscle can be visualised using calcium imaging. Combined with receptor ligands this offers a powerful means of visualising the neurotransmission process in time and space.

The next challenge will be the development of robust quantitative methodologies for the multichannel, multidimensional image volumes, which confocal systems are capable of producing.

The paper by Baker *et al*. (2003) is recommended for provoking thought about drug – receptor interactions in general as well as specifically for introducing a new tool to pharmacology, BODIPY TMR-CGP, a long-acting fluorescent β_2-adrenoceptor agonist. Other studies are starting to appear, using this compound in native tissues, where multiple cell types and multiple receptor subtypes make the issues even more complex, but perhaps the rewards will be richer (Brahmadevara *et al*., 2002; Jarajapu *et al*., 2002).

References

ARRIBAS, S.M., DOMINICZAK, A.F. & McGRATH, J.C. (1997). Reduced noradrenaline-mediated vasodilatation and β-adrenergic density in the basilar artery during ageing. *J. Vasc. Res.,* **34**(S1), 6.

ARTTAMANGKUL, S., ALVAREZ-MAUBECIN, V., THOMAS, G., WILLIAMS, J.T. & GRANDY, D.K. (2000). Binding and internalization of fluorescent opioid peptide conjugates in living cells. *Mol. Pharmacol.,* **58,** 1570 – 1580.

BAKER, J.G., HALL, I.P. & HILL, S.J. (2002). Agonist and antagonist effects of CGP 12177 on β_2-adrenoceptor-stimulated gene transcription in CHO cells transfected with the human β_2-adrenoceptor. *Br. J. Pharmacol.,* **137,** 400 – 408.

BAKER, J.G., HALL, I.P. & HILL, S.J. (2003). Pharmacology and direct visualisation of BODIPYTMR-CGP: a long acting fluorescent β_2-adrenoceptor agonist. *Br. J. Pharmacol.,* **139,** 232 – 242.

BRAHMADEVARA, N., MacDONALD, A., McGRATH, J.C. & DALY, C.J., (2002). Measurement of cellular and tissue distribution of beta-adrenoceptors in rat thoracic aorta using BODIPY-CGP 12177. *Br. J. Pharmacol.,* **137,** 93P.

BUKU, A., PRICE, J.A., MENDLOWITZ, M. & MASUR, S. (2001). Mast cell degranulating peptide binds to RBL-2H3 mast cell receptors and inhibits IgE binding. *Peptides,* **22,** 1993 – 1998.

COLEMAN, R.A., JOHNSON, M., NIALS, A.T. & VARDEY, C.J. (1996). Exosites: their current status, and their relevance to the duration of action of long-acting beta 2-adrenoceptor agonists. *Trends Pharmacol. Sci.,* **17,** 324 – 330.

DALY, C.J., MILLIGAN, C.M., MILLIGAN, G., MacKENZIE, J.F. & McGRATH, J.C. (1998). Cellular localisation and pharmacological characterisation of functioning α_1-adrenoceptors by fluorescent ligand binding and image analysis reveals identical binding properties of clustered and diffuse populations of receptors. *J. Pharmacol. Exp. Ther.,* **286,** 984 – 990.

FABRY, M., CABRELE, C., HOCKER, H. & BECK-SICKINGER, A.G. (2000). Differently labeled peptide ligands for rapid investigation of receptor expression on a new human glioblastoma cell line. *Peptides,* **21,** 1885 – 1893.

HEITHIER, H., HALLMANN, D., BOEGE, F., REILANDER, H., DEES, C., JAEGGI, K.A., ARNDT-JOVIN, D., JOVIN, T.M. & HELMREICH, E.J.M. (1994). Synthesis and properties of fluorescent β-adrenoceptor ligands. *Biochemistry,* **33,** 9126 – 9134.

HOWL, J. (1999). Fluorescent and biotinylated probes for B-2 bradykinin receptors: agonists and antagonists. *Peptides,* **20,** 515 – 518.

JACKSON, V.M. & CUNNANE, T.C. (2002). Bretylium or 6-OHDA-resistant, action potential-evoked Ca^{2+} transients in varicosities of the mouse vas deferens. *Br. J. Pharmacol.,* **135,** 1845 – 1850.

JARAJAPU, Y.P.R., MacDONALD, A., HILLIER, C., McGRATH, J.C., MacKENZIE, J.F. & DALY, C.J. (2002). Quantitative imaging of QAPB-associated fluorescence in smooth muscle cells from human skeletal muscle resistance arteries. *Br. J. Pharmacol.,* **135**(Suppl), 303P.

JENKINSON, K.M., MORGAN, J.M., FURNESS, J.B. & SOUTHWELL, B.R. (1999). Neurons bearing NK3 tachykinin receptors in the guinea-pig ileum revealed by specific binding of fluorescently labelled agonists. *Histochem. Cell Biol.,* **112,** 233 – 246.

MacKENZIE, J.F., DALY, C.J., PEDIANI, J.D. & McGRATH, J.C. (2000). Quantitative imaging in live human cells reveals intracellular α_1-adrenoceptor ligand binding sites. *J. Pharmacol. Exp. Ther.,* **294,** 434 – 443.

MADSEN, B.W., BEGLAN, C.L. & SPIVAK, C.E. (2000). Fluorescein-labeled naloxone binding to mu opioid receptors on live Chinese hamster ovary cells using confocal fluorescent microscopy. *J. Neurosci. Methods,* **97,** 123 – 131.

McGRATH, J.C. (2002). Bretylium or 6-OHDA-resistant, action potential-evoked Ca^{2+} transients in varicosities of the mouse vas deferens: commentary on Jackson and Cunnane. *Br. J. Pharmacol.,* **135,** 1841 – 1843.

McGRATH, J.C., ARRIBAS, S.M. & DALY, C.J. (1996). Fluorescent ligands for the study of receptors. *Trends Pharmacol. Sci.,* **17,** 393 – 399.

McGRATH, J.C., MacKENZIE, J.F. & DALY, C.J. (1999a). Pharmacological implications of cellular localisation of α_1-adrenoceptors in native smooth muscle cells. *J. Autonom. Pharmacol.,* **19,** 303 – 310.

McGRATH, J.C., NAGADEH, M.A., PEDIANI, J.D., MacKENZIE, J.F. & DALY, C.J. (1999b) Importance of agonists in α-adrenoceptor classification and localisation of α_1-adrenoceptors in human prostate. *Eur. Urol.,* **36**(Suppl 1), 80 – 88.

McGRATH, J.C., PEDIANI, J.D., MacMILLAN, J., MacKENZIE, J., DEIGHAN, C., WOOLHEAD, A., McGRORY, S.P., McBRIDE, M., ALI, Z., MALEKZADEH-SHAFAROUDI, M., COTECCHIA, S., ARRIBAS, S.M., VILA, E., BRIONES, A., PEREZ, D., MULLINS, J., TSUJIMOTO, G. & DALY, C.J. (2002). Adventitial cells are identified as the major location of vascular alpha 1B-adrenoceptors and may drive vascular remodelling. *Br. J. Pharmacol.,* **137,** 21P.

MILLIGAN, G. (1999). Exploring the dynamics of regulation of G protein-coupled receptors using green fluorescent protein. *Br. J. Pharmacol.,* **128,** 501 – 510.

THORLIN, T., PERSSON, P.A.I., ERIKSON, P.S., RONNBACK, L. & HANSSON, E. (2000). Astrocyte β_1-adrenergic receptor immunoreactivity and agonist induced increases in $[Ca^{2+}]_i$: differential results indicative of a modified membrane receptor. *Life Sci.,* **67,** 1285 – 1296.

TRAN, D., DURROUX, T., STELLY, N., SEYER, R., TORDJMANN, T., COMBETTES, L. & CLARET, M. (1999). Visualization of cell surface vasopressin V1a receptors in rat hepatocytes with a fluorescent linear antagonist. *J. Histochem. Cytochem.,* **47,** 401 – 409.

VANDENBULCKE, F., NOUEL, D., VINCENT, J.P., MAZELLA, J. & BEAUDET, A. (2000). Ligand-induced internalization of neurotensin in transfected COS-7 cells: differential intracellular trafficking of ligand and receptor. *J. Cell Sci.,* **113,** 2963 – 2975.

VON BOHLEN und HALBACH, O. & ALBRECHT, H.D. (2000). Identification of angiotensin IV binding sites in the mouse brain by a fluorescent binding study. *Neuroendocrinology,* **72,** 218 – 223.

(Received March 6, 2003
Revised March 14, 2003
Accepted March 18, 2003)