BJP BRITISH JOURNAL OF PHARMACOLOGY
BPS BRITISH PHARMACOLOGICAL SOCIETY

British Journal of Pharmacology (2010), 160, 1045–1047

0007-1188/10
www.brjpharmacol.org

COMMENTARY

Being mindful of seven-transmembrane receptor 'guests' when assessing agonist selectivity

Terry Kenakin

GlaxoSmithKline Research, Research Triangle Park, NC, USA

The time-honored approach of quantifying agonist selectivity through measurement of agonist affinity with binding and efficacy through potency ratios in model assays for prediction of effect in therapeutic systems can fall short of providing useful answers for functionally selective agonists. Agonists are now known to have pluridimensional efficacies that are associated with selected signalling pathways coupled to the receptor. This necessitates specifically tailored assay formats to measure predetermined efficacies of ligands to characterize agonist selectivity fully. If such assays can access signalling that directly emanates from the interaction of the agonist-bound receptor and a cytosolic signalling protein, then the Black/Leff operational model can be used to specifically quantify 'transduction ratios' (τ/K_A) that fully characterize selective activation of signalling pathways by a given agonist. As whole-cell processing of pleiotropic signalling cascades imposes cell-specific phenotypic agonist profiles, ultimately the assessment of agonist selectivity is most reliably done in the therapeutically relevant primary cell system.
British Journal of Pharmacology (2010) **160,** 1045–1047; doi:10.1111/j.1476-5381.2010.00764.x
This article is a commentary on Baker, pp. 1048–1061 of this issue. To view this paper visit http://dx.doi.org/10.1111/j.1476-5381.2010.00754.x

Keywords: receptor selectivity; agonism; functional selectivity; allosterism

Abbreviations: dimethyl-W64, N,N-bis[9-(1,3-dihydro-1,3-dioxo-4-methyl-2H-isoindol-2-yl)propyl]-N,N,N-tetramethyl-1,6-hexanediaminium diiodide; GTP, guanosine triphosphate; HEK, human embryonic kidney; NMS, N-methylscopolamine; SII, Sar^1,Ile^4,Il^8-Angiotensin II

The paper in this issue of the *BJP* by Jillian Baker (Baker, 2010) emphasizes the importance of determining both the affinity and efficacy of selective agonists in assessing overall selectivity of agonism. The value of this approach in determining agonist receptor selectivity is clear, as agonist potency can be controlled by affinity or efficacy, or both. This basic pharmacological principle can be extended in light of our present knowledge of pleiotropic signalling through seven transmembrane receptors (7TMRs). Specifically, it is apparent that agonists activating receptors coupled to multiple signalling proteins can produce biased activation of some signalling pathways over others (see Kenakin, 2006; Mailman, 2007). Under these circumstances, conventional whole-cell measures of affinity (binding) and efficacy (whole-cell potency ratios) can be misleading. It is therefore worth discussing the concept of agonist selectivity in the functionally selective world.

7TMRs are allosteric systems mediating the vectorial transduction of energy from one locus on the receptor to another.

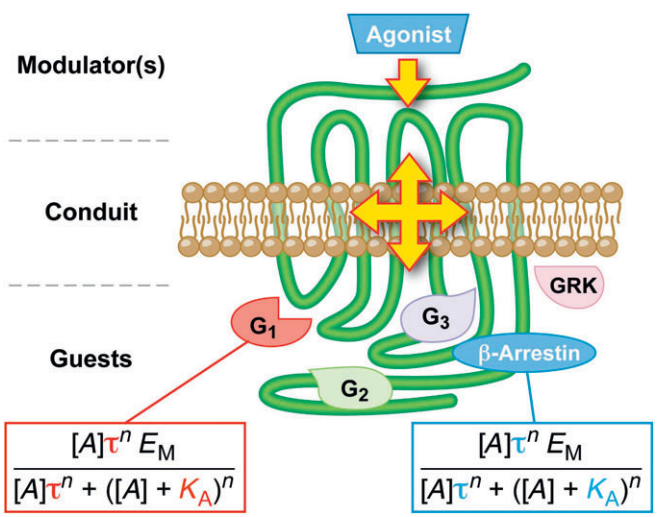

Figure 1 Classical allosteric system for 7TMR agonism. All agonists become the 'modulator', the receptor is the 'conduit' and the cytosolic signalling molecules are the 'guests'. Within this scheme, the Black/Leff operational model can be used to quantify selective activation of pathways through identification of unique $\log(\tau/K_A)$ ratios.

Correspondence: Terry Kenakin, Molecular Discovery Research, GlaxoSmithKline Research and Development, 5 Moore Drive, Research Triangle Park, NC 27709, USA. E-mail: terry.p.kenakin@gsk.com
Received 15 February 2010; revised 26 February 2010; accepted 1 March 2010

It is useful to think of 7TMR selective agonism in terms of classical allosteric terminology, thus, agonists are *modulators*, the receptor protein the *conduit* and cytosolic signalling proteins the *guests* (see Figure 1). It should be noted that considering the separate binding loci of agonists and signalling proteins, *all* agonists, whether they bind to the natural endogenous agonist binding site or other binding sites, are allosteric modulators with respect to the signalling protein. In addition, the terms modulator and guest are interchangeable in terms of energy flow. Thus, for an agonist as a modulator, the guest may be a G-protein. However, when viewed from the cytosol, the modulator could also be the G-protein and the agonist the guest; both viewpoints are pharmacologically relevant (*vide infra*). The receptor can be seen as rolling on an energy landscape as it adopts multiple conformations in response to changes in energy of the system; the various energy wells on this landscape represent favoured receptor conformations, some of which may mediate cellular signalling (Fraunfelder *et al*., 1991; Onaran *et al*., 2002; Hilser *et al*., 2006). As receptors take conformational excursions away from canonical native structures, the addition of an agonist modulator will necessarily bias the sampled conformations of the receptor and produce a new energy landscape for it to traverse. Under these circumstances, an allosteric modulator essentially produces a 'new receptor' with potentially different capabilities to interact with cytosolic signalling proteins. For example, a molecule may stabilize a conformation that favourably binds to and activates a G-protein, and thus induces visible agonism. A new energy landscape may not contain conspicuous energy wells for binding to standard signalling molecules, but rather may bias the receptor towards conformations mediating other behaviours such as in cases where non-G-protein signalling antagonists actively internalize receptors (Roth and Chuang, 1987) or only produce extracellular signal-regulated kinase (ERK) signalling through β-arrestin (see Azzi *et al*., 2003). These effects blur distinctions between classical 'agonist' and 'antagonist' labels for molecules (Kenakin, 2008). Thus, a given molecule can have numerous efficacies (pluridimensional efficacy, Galandrin and Bouvier, 2006), and this leads to what is commonly termed functional selectivity or agonist 'bias'.

The nature of the guest molecule dictates the strength and the type of signal produced by an agonist, but it also determines the affinity of the modulator agonist for the receptor. As mentioned previously, allosteric energy has reciprocal vectorial properties. Thus, if a molecule increases the affinity of the receptor for a given guest (i.e. G-protein), the same is true in the reverse direction, namely the guest will increase the affinity of the receptor for the modulator. An example of this effect has been shown experimentally with [^{3}H]-dimethyl-W84 and [^{3}H]-NMS binding on muscarinic M_2 receptors (Trankle *et al*., 1999). The influence of different coupling molecules (i.e. G-proteins) on the affinity of the muscarinic receptor for the agonist carbachol was elegantly demonstrated by Birdsall *et al*. (1980) in the form of a 56-point [^{3}H]-propylbenzylcholine displacement by the agonist carbachol curve spanning seven orders of magnitude. The different affinities of carbachol with each G-protein couple are made manifest by the extremely broad range of the total binding curve demonstrating the fact that different guests can confer different affinities on the receptor for the agonist. For this reason, functional selectivity must be described in terms of the affinity (and activating properties) of the agonist-bound receptor for the signalling protein *and* the affinity of the receptor for the agonist. The identity of the guest can be extremely important to the interaction of the agonist with the receptor. For example, the modulator eburnamonine binds to the muscarinic M_2 receptor; if the guest molecule is acetylcholine, the binding is enhanced by a factor of 15. If the guest molecule is pilocarpine, the affinity is *decreased* by a factor of 25 (Jakubic *et al*., 1997). For these reasons, the signalling pathway activated by the agonist-bound receptor also controls the affinity of the receptor for the agonist.

A most useful tool for characterization of agonism is the Black/Leff operational model (Black and Leff, 1983), as it involves both affinity (K_A, the equilibrium dissociation constant of the agonist–receptor complex) and efficacy (K_E, the virtual equilibrium constant for the complex formed between the 'activated' receptor and cellular stimulus–response machinery). This latter term embodies both tissue sensitivity and the intrinsic efficacy of the agonist in the form of τ which is the receptor density divided by K_E. Therefore, selective activation of a pathway can be described by both K_A and τ; a convenient single parameter to do this is $\log(\tau/K_A)$ (Kenakin, 2009). As shown in Figure 1, if the activated (agonist-bound) receptor directly interacts with a range of signalling proteins, then different cellular signals may result each quantified by unique τ/K_A ratios. The difficulty comes in accessing these selective activations to characterize 'selective' agonism with tools that measure macro-affinity (i.e. total agonist binding) and cell-based efficacy (which may be a mixture of pathways emphasized in agonist-specific ways). Under these circumstances, conventional methods to measure affinity (i.e. binding) or selective efficacy (agonist potency ratios from whole-cell response) become blunt instruments in dissecting pathway selectivity. Radioligand binding of agonist–receptor interaction in membranes may result in measures of a 'high-affinity' state which may be an amalgam of receptor–G-protein complexes, only one of which may be relevant to a given signalling pathway. For instance, the affinity of [^{125}I]-human calcitonin for the human calcitonin receptor is 400-fold greater than the EC_{50} for calcium transient response in HEK cells; presumably, the known pleiotropic interaction of the calcitonin receptor with G_i, G_q and G_s protein, and the absence of GTP in the binding assay contribute to the high-affinity binding that may not be relevant for functional response (Watson *et al*., 2000). Similarly, total cellular response may be a crude indicator of agonist–receptor signalling. For instance, activation of ERK1/2 can be achieved through both G-protein and β-arrestin (non-G-protein) routes. Thus, for angiotensin receptors, equiactive ERK responses to angiotensin II (G-protein + β-arrestin activation) and the analog SII (only β-arrestin activation) belie the separate signalling pathways these ligands may activate in any given system (Wei *et al*., 2003).

The observation of functionally selective agonism has considerably muddied the waters with respect to quantifying relative potency in one system and assuming that it will be applicable to all systems. This is because the cell can influence agonist activity by virtue of modifying the relative stoichiom-

etry of signalling components, that is, a β-arrestin-dependent agonist will be more active in cells with high levels of β-arrestin, as opposed to those with lower levels of β-arrestin. Two potential (and concurrent) strategies to address this problem could be envisioned. The first may be to characterize therapeutically relevant selectivity in the therapeutically relevant primary cell, perhaps through label-free technology. A second may be to quantify agonist bias through the operational model with systems that mediate signalling directly interacting with the agonist-bound receptor, and relate this scale to therapeutically observed phenotypic agonist activity. While, ultimately, this will not furnish direct measures of therapeutically relevant selectivity, it may furnish a scale through which medicinal chemists may actively optimize useful biased activity.

Conflict of interest

The author declares no conflict of interest in this work.

References

Azzi M, Charest PG, Anger S, Rousseau G, Kohout T, Bouvier M (2003). β-Arrestin-mediated activation of MAPK by inverse agonists reveals distinct active conformations for G-protein-coupled receptors. *Proc Natl Acad Sci USA* **100**: 11406–11411.

Baker J (2010).The selectivity of β-adrenoceptor agonists at the human β1, β2 and β3 adrenoceptors. *Br J Pharmacol* **160**: 1048–1061.

Birdsall NJM, Hulme EC, Burgen ASV (1980). The character of the muscarinic receptors in different regions of the rat brain. *Proc R Soc Lond B Biol Sci* **207**: 1–12.

Black JW, Leff P (1983). Operational models of pharmacological agonism. *Proc R Soc Lond B Biol Sci* **220**: 141–162.

Fraunfelder H, Sligar SG, Wolynes PG (1991). The energy landscapes and motions of proteins. *Science* **254**: 1598–1603.

Galandrin S, Bouvier M (2006). Distinct signaling profiles of β_1 and β_2 adrenergic receptor ligands toward adenylyl cyclase and mitogen-activated protein kinase reveals the pluridimensionality of efficacy. *Mol Pharmacol* **70**: 1575–1158.

Hilser VJ, Garcia-Moreno EB, Oas TG, Kapp G, Whitten ST (2006). A statistical thermodynamic model of protein ensembles. *Chem Rev* **106**: 1545–1558.

Jakubic J, Bačáková L, El-Fakahany EE, Tuček S (1997). Positive cooperativity of acetylcholine and other agonists with allosteric ligands on muscarinic acetylcholine receptors. *Mol Pharmacol* **52**: 172–179.

Kenakin TP (2006). Collateral efficacy as pharmacological problem applied to new drug discovery. *Expert Opin Drug Discov* **1**: 635–652.

Kenakin TP (2008). Pharmacological onomastics: what's in a name? *Br J Pharmacol* **153**: 432–438.

Kenakin TP (2009). Putting numbers to shapeshifting proteins. *Trends Pharmacol Sci* **30**: 460–469.

Mailman RB (2007). GPCR functional selectivity has therapeutic impact. *Trends Pharmacol Sci* **28**: 390–396.

Onaran HO, Scheer A, Cotecchia S, Costa T (2002). A look at receptor efficacy. From the signaling network of the cell to the intramolecular motion of the receptor. In: Kenakin TP, Angus JA (eds). *The Pharmacology of Functional, Biochemical, and Recombinant Systems Handbook of Experimental Pharmacology*, Vol. 148. Springer: Heidelberg, pp. 217–280.

Roth BL, Chuang D-M (1987). Multiple mechanisms of serotonergic signal transduction. *Life Sci* **41**: 1051–1064.

Trankle C, Weyand A, Schroter A, Mohr K (1999). Using a radioalloster to test predictions of the cooperativity model for gallamine binding to the allosteric site of muscarinic acetylcholine (m2) receptors. *Mol Pharmacol* **56**: 962–965.

Watson C, Chen G, Irving PE, Way J, Chen W-J, Kenakin TP (2000). The use of stimulus-biased assay systems to detect agonist-specific receptor active states: implications for the trafficking of receptor stimulus by agonists. *Mol Pharmacol* **58**: 1230–1238.

Wei H, Ahn S, Shenoy SK, Karnik SS, Hunyady L, Luttrell LM *et al*. (2003). Independent beta-arrestin 2 and G protein mediated pathways for angiotensin II activation of extracellular signal-regulated kinases 1 and 2. *Proc Natl Acad Sci USA* **100**: 10782–10787.