

MOLPHARM/2005/013060

What's for lunch at the conformational cafeteria?

William P. Clarke

Department of Pharmacology, University of Texas Health Science Center, San Antonio, TX.
78229-3900 USA.

Running Title: Ligand-selective receptor conformations

Corresponding author:

William P. Clarke
Department of Pharmacology – MS #7764
University of Texas Health Science Center
7703 Floyd Curl Drive
San Antonio, TX 78229-3900
Voice: (210) 567-4171
Fax: (210) 567-6952
Email: clarkew@uthscsa.edu

Number of abstract words	101
Number of text pages	6
Number of tables:	0
Number of figures:	0
Number of references:	33
Number of words	1539

Abstract

In this issue of Molecular Pharmacology, Mukhopadhyay and Howlett present evidence for ligand-selective conformations of the CB1 cannabinoid receptor with differential coupling to G proteins. Ligand-directed signaling to different cellular effector pathways extends drug selectivity beyond that afforded by differential affinity for different receptor subtypes. The challenge for pharmacologists of the future will be not only to identify ligand-selective receptor conformations, but to develop an understanding of the relationship between those conformations, cell function, and ultimately therapeutics. As we learn more about ligand-selective receptor conformations, it should be possible to develop response-selective drugs which maximize therapeutic efficacy and minimize unwanted effects.

Since the birth of the discipline of Pharmacology, one of the primary goals of pharmacologists has been to reveal the messages contained within drug molecules that influence system physiology. For many years it was thought that messages contained within drugs were delivered by two drug properties: affinity (the capacity to bind) and intrinsic efficacy (the capacity to change a receptor's behavior toward its host). Affinity was responsible for a drug's selectivity (which receptors a drug could influence). Intrinsic efficacy conveyed a drug's strength by the magnitude (null, weak {partial}, strong) and direction (negative or positive) of the receptor stimulus it produced upon binding.

Intrinsic efficacy was originally proposed by Furchgott (1966) to reflect the capacity of a drug to produce a receptor stimulus (Stephenson, 1956). This receptor stimulus was delivered to the signal transduction apparatus of a cell, resulting in a cellular response, the magnitude of which was dependent upon the size of the total stimulus (the product of the receptor stimulus and the number of receptors in the cell) and the efficiency of stimulus-response coupling (signal transduction). In Furchgott's framework, intrinsic efficacy was a drug property, unique for each drug-receptor pair, that was independent of the signaling system coupled to a receptor. Although intrinsic efficacy could not be measured directly, relative efficacy measures, which normalize for differences in signal transduction efficiency, could be used to assess the relative magnitude of the stimulus a drug elicits. Thus, intrinsic efficacy was of value for drug discovery since it could be used to predict the relative magnitude of a response to a drug in any cell/tissue if the drug's relative efficacy in another tissue was known.

In more contemporary models of receptor function, intrinsic efficacy and receptor stimulus can be related to the capacity of a drug to promote a change in receptor conformation

which increases its ability to interact with cellular signaling molecules, such as G proteins. For receptors coupled to multiple signaling pathways within a cell, a single receptor stimulus would be delivered to each of the signaling pathways, each of which could have different transduction efficiencies to convert the stimulus into a response. However, because there is a single stimulus, measurement of drug relative efficacy (which obviates response-dependent differences in signaling efficiency) must be the same for each response coupled to a receptor.

In recent years, data have accumulated which challenge this view of intrinsic efficacy. Several studies have reported that agonist relative efficacy is different when different responses are measured, even within the same cell (for reviews see, Clarke and Bond, 1998; Kenakin, 2003a; Kenakin, 2003b). Such studies have initiated a re-definition of the concept of intrinsic efficacy such that ligands can produce multiple stimuli (have multiple intrinsic efficacies) upon interaction with a receptor and can differentially regulate each of multiple signaling pathways coupled to a receptor. This ligand behavior has been termed “agonist-directed trafficking of receptor stimulus”, “functional selectivity”, “stimulus trafficking”, and “biased agonism”. The underlying mechanism for this is proposed to be based upon the capacity of ligands to promote the unique, ligand-selective receptor conformations which have differential efficacy to regulate signal transduction pathways.

Receptors, like all proteins, spontaneously adopt a variety of conformations, some of which may be able to regulate signaling pathways and are thus said to be “active” and given the symbols, R^* , R^{**} , R^{***} , etc., with R denoting an inactive receptor. When a ligand is added, it will bind to these receptor conformations according to the relative affinity of the ligand for each conformation and thus will enrich certain receptor conformations and deplete others. A different

ligand, with different relative affinities, will stabilize a different spectrum of receptor conformations. Kenakin has coined the term “conformational cafeteria” to describe the process whereby ligands enter receptor space and selectively stabilize (“choose”) certain conformations for which they have highest affinity (Kenakin, 2002; Kenakin and Onaran, 2002). As a consequence, ligand-selective receptor conformations may mediate ligand-selective signaling via a single receptor subtype.

In this issue of *Molecular Pharmacology*, Mukhopadhyay and Howlett (2005) provide evidence of ligand-selective conformations of the CB1 cannabinoid receptor. Using CHAPS solubilized extracts of membranes prepared from N18TG2 cells, which naturally express the CB1 receptor, Mukhopadhyay and Howlett examined the ability of three structurally different classes of CB1 ligands, previously characterized as agonists, to interact with specific G protein subtypes ($G\alpha_i1$, $G\alpha_i2$, and $G\alpha_i3$). In the absence of ligands, a large fraction of the solubilized $G\alpha_i$ proteins co-immunoprecipitated with the CB1 receptor in a pertussis toxin- and $GTP\gamma S$ -sensitive manner, confirming earlier reports that the CB1 receptor can spontaneously couple to and activate G proteins (constitutive activity) in recombinant and native cell systems (for a review see, Pertwee, 2005). In the absence of $GTP\gamma S$, the aminoalkylindole WIN55212-2 (WIN), the cannabinoid desacetyllevonantradol (DALN), or the eicosanoid (R)-methanandamide promoted a mixture of CB1 receptor- $G\alpha_i$ complexes and free receptors differentially depending upon the $G\alpha_i$ subtype. These data suggest that there is differential G protein subtype coupling to the CB1 receptor when occupied by different ligands.

The effect of $GTP\gamma S$ to destabilize receptor-G protein complexes was examined in the presence of the three different ligands. As expected, incubation with $GTP\gamma S$ reduced (85-100%)

the quantity of G protein that co-immunoprecipitated with the CB1 receptor. The ability of GTP γ S to promote G protein dissociation was affected differentially, depending upon the G protein subtype and the ligand used. For example, whereas WIN did not alter GTP γ S-promoted dissociation of G α_i 1, G α_i 2 or G α_i 3, DALN completely prevented GTP γ S-mediated dissociation of G α_i 3, had little effect on dissociation of G α_i 2 and partially reduced dissociation of G α_i 1. The pattern of G protein subtype effects produced by (R)-methanandamide differed from those of WIN and DALN. In addition there were potency differences between G protein subtypes for the ligands to influence GTP γ S-mediated dissociation. Taken together, such data are not consistent with a single active receptor conformation which interacts with G proteins and suggest instead that ligands are able to promote unique conformations with different abilities to interact with different G protein subtypes.

Interestingly, the effects of DALN on G α_i 3 and (R)-methanandamide on G α_i 1 and G α_i 2 were similar to the effect produced by the prototypical inverse agonist SR141716. These results suggest that DALN and (R)-methanandamide are protean ligands; agonists for some G protein subtypes and inverse agonists for others. Such differential activation of G α_i protein subtypes may have physiological relevance. Recently Holstein et al. (2004) reported that ERK1/2 activation by the calcium-sensing receptor, stimulated with 4 mM calcium, was mediated by G α_i 2 but not by G α_i 1 or G α_i 3. These data suggest that even though they are highly homologous, the different G α_i subtypes may subserve different physiological functions in cells. Consequently, differential activation/inactivation of these G protein subtypes by cannabinoid ligands may lead to different physiological effects.

In addition to differential G protein coupling and signaling (Berg et al., 2001; Berg et al.,

1998; Bonhaus et al., 1998; Cordeaux et al., 2000; Kurrasch-Orbaugh et al., 2003; MacKinnon et al., 2001; Mailman and Gay, 2004; Mottola et al., 2002), there have been a variety of other approaches which provide evidence for ligand-selective receptor conformations, including ligand-dependent receptor internalization (Hunyady et al., 1994; Roettger et al., 1997; Whistler et al., 1999), phosphorylation and desensitization (Blake et al., 1997; Chakrabarti et al., 1998; Stout et al., 2002; Thomas et al., 2000), ligand binding affinity (Liapakis et al., 2004; Lopez-Gimenez et al., 2001), and kinetics of activation (Krumins and Barber, 1997; Swaminath et al., 2004). Perhaps the most direct method is through the use of fluorescent receptor tags which are sensitive to changes in receptor conformation. Using fluorescence lifetime spectroscopy, Ghanouni et al. (2001) showed that isoproterenol and dobutamine produced different conformational populations of the solubilized β_2 -adrenergic receptor labeled with fluorescein maleimide on an environmentally sensitive cysteine located in the third intracellular loop. Recently a new cysteine-reactive fluorescent probe (aminophenoxazone maleimide, APM) has been developed with better spectral characteristics which should prove useful in measuring ligand-dependent conformational changes in receptors (Cohen et al., 2005). In addition, exciting new developments in Fluorescent Resonance Energy Transfer (FRET) using a small membrane-permeable fluorescein derivative (fluorescein arsenical hairpin binder, FAsH), which does not interfere with signaling of the human adenosine A₂ receptor to adenylyl cyclase, should allow sensitive detection of ligand-dependent conformational changes in living cells (Hoffmann et al., 2005).

Although new techniques and chemical probes will likely permit us to distinguish ligand-selective receptor conformational populations with higher resolution, the challenge of assessing the functional relevance of those conformations remains. Even if we could map the three

MOLPHARM/2005/013060

dimensional locations of each atom in a receptor occupied with different ligands, we still need to know how much of a difference (in conformation) makes a difference. It is important to establish how ligand-selective receptor conformations interact differentially with signaling molecules, such as G proteins, as was done by Mukhopadhyay and Howlett (2005). These authors showed that different cannabinoids cause the CB1 receptor to interact differentially with G proteins, suggesting that CB1 ligands may promote different physiological, and possibly therapeutic, responses. Thus, the challenge for pharmacologists in the future will be not only to identify ligand-selective receptor conformations, but to develop an understanding of the relationship between those conformations, cell function, and ultimately therapeutics. As this understanding develops, the hope for response-selective drugs with improved therapeutic selectivity may be realized.

References

- Berg KA, Cropper JD, Niswender CM, Sanders-Bush E, Emeson RB and Clarke WP (2001) RNA-editing of the 5-HT_{2C} receptor alters agonist-receptor-effector coupling specificity. *British Journal of Pharmacology* **134**:386-392.
- Berg KA, Maayani S, Goldfarb J, Scaramellini C, Leff P and Clarke WP (1998) Effector pathway-dependent relative efficacy at serotonin type 2A and 2C receptors: Evidence for agonist-directed trafficking of receptor stimulus. *Mol. Pharmacol.* **54**:94-104.
- Blake AD, Bot G, Freeman JC and Reisine T (1997) Differential opioid agonist regulation of the mouse mu opioid receptor. *J Biol Chem* **272**:782-790.
- Bonhaus DW, Chang LK, Kwan J and Martin GR (1998) Dual activation and inhibition of adenylyl cyclase by cannabinoid receptor agonists: evidence for agonist-specific trafficking of intracellular responses. *J Pharmacol Exp Ther* **287**:884-888.

Chakrabarti S, Law PY and Loh HH (1998) Distinct differences between morphine- and [D-Ala²,N-MePhe⁴,Gly-ol⁵]-enkephalin-mu-opioid receptor complexes demonstrated by cyclic AMP-dependent protein kinase phosphorylation. *J Neurochem* **71**:231-239.

Clarke WP and Bond RA (1998) The elusive nature of intrinsic efficacy. *Trends Pharmacol. Sci.* **19**:270-276.

Cohen BE, Pralle A, Yao X, Swaminath G, Gandhi CS, Jan YN, Kobilka BK, Isacoff EY and Jan LY (2005) A fluorescent probe designed for studying protein conformational change. *Proc Natl Acad Sci U S A* **102**:965-970.

Cordeaux Y, Briddon SJ, Megson AE, McDonnell J, Dickenson JM and Hill SJ (2000) Influence of receptor number on functional responses elicited by agonists acting at the human adenosine A(1) receptor: Evidence for signaling pathway-dependent changes in agonist potency and relative intrinsic activity. *Molecular Pharmacology* **58**:1075-1084.

Furchgott RF (1966) The use of β -haloalkylamines in the differentiation of receptors and in the determination of dissociation constants of receptor-agonist complexes., in *Advances in Drug Research* (Harper NJ and Simmonds AB eds) pp 21-55, Academic Press, London.

Ghanouni P, Gryczynski Z, Steenhuis JJ, Lee TW, Farrens DL, Lakowicz JR and

Kobilka BK (2001) Functionally different agonists induce distinct conformations in the G protein coupling domain of the beta 2 adrenergic receptor. *J Biol Chem* **276**:24433-24436.

Hoffmann C, Gaietta G, Bunemann M, Adams SR, Oberdorff-Maass S, Behr B,

Vilardaga JP, Tsien RY, Ellisman MH and Lohse MJ (2005) A FLAsH-based FRET approach to determine G protein-coupled receptor activation in living cells. *Nature Meth.* **2**:171-176.

Holstein DM, Berg KA, Leeb-Lundberg LM, Olson MS and Saunders C (2004)

Calcium-sensing receptor-mediated ERK1/2 activation requires G α hi2 coupling and dynamin-independent receptor internalization. *J Biol Chem* **279**:10060-10069.

Hunyady L, Baukal AJ, Balla T and Catt KJ (1994) Independence of type I

angiotensin II receptor endocytosis from G protein coupling and signal transduction. *J Biol Chem* **269**:24798-24804.

Kenakin T (2002) Drug efficacy at G protein-coupled receptors. *Annual Review of Pharmacology and Toxicology* **42**:349-379.

Kenakin T (2003a) A guide to drug discovery: Predicting therapeutic value in the lead optimization phase of drug discovery. *Nat Rev Drug Discov* **2**:429-438.

Kenakin T (2003b) Ligand-selective receptor conformations revisited: the promise and the problem. *Trends in Pharmacological Sciences* **24**:346-354.

Kenakin T and Onaran O (2002) The ligand paradox between affinity and efficacy: can you be there and not make a difference? *Trends Pharmacol Sci* **23**:275-280.

Krumins AM and Barber R (1997) The stability of the agonist beta2-adrenergic receptor-Gs complex: evidence for agonist-specific states. *Mol Pharmacol* **52**:144-154.

Kurrasch-Orbaugh DM, Watts VJ, Barker EL and Nichols DE (2003) Serotonin 5-hydroxytryptamine(2A) receptor-coupled phospholipase C and phospholipase A(2) signaling pathways have different receptor reserves. *Journal of Pharmacology and Experimental Therapeutics* **304**:229-237.

Liapakis G, Chan WC, Papadokostaki M and Javitch JA (2004) Synergistic contributions of the functional groups of epinephrine to its affinity and efficacy at the beta2 adrenergic receptor. *Mol Pharmacol* **65**:1181-1190.

Lopez-Gimenez JF, Villazon M, Brea J, Loza MI, Palacios JM, Mengod G and Vilaro

MT (2001) Multiple conformations of native and recombinant human 5-hydroxytryptamine(2a) receptors are labeled by agonists and discriminated by antagonists. *Mol Pharmacol* **60**:690-699.

MacKinnon AC, Waters C, Jodrell D, Haslett C and Sethi T (2001) Bombesin and substance P analogues differentially regulate G-protein coupling to the bombesin receptor. Direct evidence for biased agonism. *J Biol Chem* **276**:28083-28091.

Mailman RB and Gay EA (2004) Novel mechanisms of drug action: Functional selectivity at D-2 dopamine receptors (A lesson for drug discovery). *Medicinal Chemistry Research* **13**:115-126.

Mottola DM, Kilts JD, Lewis MM, Connery HS, Walker QD, Jones SR, Booth RG, Hyslop DK, Piercey M, Wightman RM, Lawler CP, Nichols DE and Mailman RB (2002) Functional selectivity of dopamine receptor agonists. I. Selective activation of postsynaptic dopamine D-2 receptors linked to adenylate cyclase. *Journal of Pharmacology and Experimental Therapeutics* **301**:1166-1178.

Mukhopadhyay S and Howlett AC (2005) Chemically distinct ligands promote differential CB1 cannabinoid receptor-Gi protein interactions. *Mol Pharmacol*.

Pertwee RG (2005) Inverse agonism and neutral antagonism at cannabinoid CB1 receptors. *Life Sci* **76**:1307-1324.

Roettger BF, Ghanekar D, Rao R, Toledo C, Yingling J, Pinon D and Miller LJ (1997) Antagonist-stimulated internalization of the G protein-coupled cholecystokinin receptor. *Mol Pharmacol* **51**:357-362.

Stephenson RP (1956) A modification of receptor theory. *Br. J. Pharmacol.* **11**:379-393.

Stout BD, Clarke WP and Berg KA (2002) Rapid desensitization of the serotonin(2C) receptor system: effector pathway and agonist dependence. *J Pharmacol Exp Ther* **302**:957-962.

Swaminath G, Xiang Y, Lee TW, Steenhuis J, Parnot C and Kobilka BK (2004) Sequential binding of agonists to the beta2 adrenoceptor. Kinetic evidence for intermediate conformational states. *J Biol Chem* **279**:686-691.

Thomas WG, Qian H, Chang CS and Karnik S (2000) Agonist-induced phosphorylation of the angiotensin II (AT(1A)) receptor requires generation of a conformation that is distinct from the inositol phosphate-signaling state. *J Biol Chem* **275**:2893-2900.

Whistler JL, Chuang HH, Chu P, Jan LY and von Zastrow M (1999) Functional dissociation of mu opioid receptor signaling and endocytosis: implications for the biology of opiate tolerance and addiction. *Neuron* **23**:737-746.