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What's for lunch at the conformational cafeteria?

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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 it's 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 GTPγS-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 GTPγS, 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 GTPγS to destabilize receptor-G protein complexes was examined in the presence of the three different ligands. As expected, incubation with GTPγS reduced (85-100%)

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the quantity of G protein that co-immunoprecipitated with the CB1 receptor. The ability of GTPγS 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 GTPγS-promoted dissociation of $G\alpha_i1$, $G\alpha_i2$ or $G\alpha_i3$, DALN completely prevented GTPγS-mediated dissociation of $G\alpha_i3$, had little effect on dissociation of $G\alpha_i2$ and partially reduced dissociation of $G\alpha_i1$. 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 GTPγS-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 $G\alpha_i3$ and (R)-methanandamide on $G\alpha_i1$ and $G\alpha_i2$ 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 $G\alpha_i$ 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 $G\alpha_i2$ but not by $G\alpha_i1$ or $G\alpha_i3$. These data suggest that even though they are highly homologous, the different $G\alpha_i$ 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 ß2-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, FlAsH), 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

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.

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