

PERSPECTIVE

Ligand-Directed Signaling: 50 Ways to Find a Lover

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ABSTRACT

In contrast to earlier concepts, it seems that distinct ligands acting on the same receptor may elicit qualitative different response patterns, a phenomenon given many names, including “functional selectivity,” “agonist-directed trafficking,” “biased agonism,” “protean agonism,” or “ligand-directed signaling.” In this issue of *Molecular Pharmacology*, Sato et al. (p. 1359) extend this concept to β_3 -adrenergic receptors and re-

port that distinct ligands can activate a single distal response via different signaling pathways. Moreover, they demonstrate that expression density can affect how distinct ligands acting on the same receptor differentially induce cellular responses. We discuss the underlying concepts for such findings and their implications for drug discovery.

Traditional pharmacological concepts have assumed that all ligands activating a given receptor elicit the same response pattern and that they differ only quantitatively (i.e., in potency and/or efficacy). However, recent evidence from a variety of G-protein-coupled receptors (GPCRs) has demonstrated that agonists acting on the same receptor can induce differential cellular response patterns. Such evidence has been obtained, for example, for serotonin, opioid, vasopressin, dopamine, and β -adrenergic receptors (Gilchrist, 2007; Urban et al., 2007). This phenomenon has been reported under a variety of names including “functional selectivity,” “agonist-directed trafficking,” “biased agonism,” “protean agonism,” or “ligand-directed signaling” (LDS). In each case, the basic observation supporting this concept has been that two or more agonists differ in their order of potency and/or efficacy between responses mediated by the same receptor. To unequivocally demonstrate that these differential responses indeed are mediated via the same receptors, such studies have largely been carried out using cloned and heterologously transfected receptors, but it is very likely that

similar phenomena occur with endogenously expressed receptors (Lauckner et al., 2005).

In this issue of *Molecular Pharmacology* Sato et al. (2007) report on β_3 -adrenergic receptors transfected into Chinese hamster ovary cells to further explore LDS. They compared several responses in cells expressing a low density of β_3 -adrenergic receptors, including cAMP formation, extracellular acidification rates (ECAR), and activation of the protein kinase p38. For cAMP formation, isoproterenol and CL316243 were full agonists, whereas SR59230A was an antagonist; for ECAR, all three were full agonists; and for p38 activation, SR59230A was more efficacious than CL316243. These differences in order of efficacy establish LDS at β_3 -adrenergic receptors.

However, two other observations are even more noteworthy. First, the authors show that ECAR stimulation by isoproterenol and CL316243 is mediated by both cAMP and p38, whereas p38 is the sole mediator of the ECAR response to SR59230A, demonstrating that agonists may activate a single cellular response (e.g., ECAR) by distinct signal transduction pathways (Fig. 1). The second key observation of Sato et al. (2007) is that the expression density of the β_3 -adrenergic receptor affected LDS. Thus, in confirmation of previous findings (Hutchinson et al., 2006), expressing a high density of receptors turned SR59230A from an antagonist to a partial

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ABBREVIATIONS: GPCR, G-protein-coupled receptor; LDS, ligand-directed signaling; ECAR, extracellular acidification rate; CL316243, (*R,R*)-5-[2-[[2-(3-chlorophenyl)-2-hydroxyethyl]-amino]-propyl]1,3-benzodioxole-2,2-decarboxylate; SR59230A, 3-(2-ethylphenoxy)-1-[(1*S*)-1,2,3,4-tetrahydronaphth-1-ylamino]-2*S*-2-propanol oxalate; MAPK, mitogen-activated protein kinase.

agonist for cAMP formation. In these high expressing cells, cAMP was the main pathway mediating ECAR for all three agonists. These findings confirm predictions on the impact of expression density on LDS by computer modeling studies (Kinzer-Ursem and Linderman, 2007).

More Than Two Receptor States?

In recent years, we had assumed that GPCRs exist in an inactive state R and an active state R* (Perez and Karnik, 2005). Whereas inverse agonists shift the equilibrium between the two states toward R, agonists shift it toward R*. The concept of LDS implies that actually more than one R* exists. This is not surprising given that site-directed mutagenesis studies suggest that different ligands use overlapping but distinct binding pockets within a given GPCR (Sato et al., 1999). Accordingly, molecular modeling predicts that chemically distinct agonists induce somewhat different receptor conformations (Blin et al., 1993). This possibility of distinct receptor conformations being induced by various agonists is directly supported by fluorescence resonance energy transfer studies (Granier et al., 2007; Kobilka and Deupi, 2007). Similar studies with inverse agonists also support the idea that not all ligands will induce the same receptor conformation (Rochais et al., 2007).

The concept of different ligand-induced receptor conformations as the structural basis of LDS also touches on the concept of allosteric receptor modulation. While "allosteric" literally means that a ligand binds to a site on a receptor that is topographically distinct from the orthosteric site occupied by the endogenous agonist, allosteric modulation has largely been interpreted as conformational change of a receptor induced by an allosteric ligand, which affects the binding of orthosteric agonists (Leach et al., 2007). Hence, allosteric modulators may differentially affect the ability of chemically distinct agonists to activate a receptor and elicit cellular response patterns (Schwartz and Holst, 2007). Moreover, some receptor conformations may be more susceptible to allosteric modulation than others (Maillet et al., 2007), adding another layer of complexity to the phenomenon of LDS.

If we further assume that the receptor/G-protein interaction, like every biomolecular interaction, depends on the mutual affinities and the relative quantities of the participating

molecules, it becomes obvious that distinct receptor conformations may relatively favor different G-proteins, at least in cases where a given receptor can activate more than one G-protein subtype or family. This also implies that a greater degree of LDS can be expected for receptors promiscuously coupling to multiple G-proteins than for those that faithfully couple to a single G-protein. Further complexity of this scheme arises in the case of receptors with coding region polymorphisms, which may affect the ability of the receptor to assume certain conformations (Rochais et al., 2007). Moreover, it is not unlikely that G-proteins also have more than one active and one inactive conformation, and hence that distinct receptor conformations could induce differential G-protein conformations, which again might favor coupling to distinct effector molecules (e.g., adenylyl cyclase versus certain types of ion channels).

Although the concept of LDS until now has mainly been interpreted in the context of differential G-protein coupling, distinct receptor conformations are also likely to affect the ability of a GPCR to directly interact with other molecules potentially involved in signal transduction (e.g., arrestins) (DeWire et al., 2007; Premont and Gainetdinov, 2007; Violin and Lefkowitz, 2007). This is of specific interest in the context of the study by Sato et al. (2007), because arrestins play an important role in coupling GPCR activation to protein kinases such as p38 (DeWire et al., 2007; Premont and Gainetdinov, 2007). Moreover, this concept is not necessarily limited to GPCR but may also be applicable to other receptors that involve bimolecular interactions as part of their signaling (e.g., receptors recruiting tyrosine kinases and perhaps even ligand-activated transcription factors).

Diverging and Converging Signaling Pathways

Whereas the ideas above can explain why multiple agonists acting on the same receptor may differentially activate G-proteins or other proximal signaling molecules, they do not directly explain why they should elicit the same response by different pathways. However, not only do cells have to exhibit complex responses to a single stimulus [requiring divergence of signaling (i.e., coupling of one proximal to multiple distal signaling molecules)], they also have to integrate the information from various concomitant stimuli [requiring convergence of signaling (i.e., multiple proximal signaling molecules funneling into a single distal effector molecule)]. For example, p38 can be activated by at least three mitogen-activated protein kinase (MAPK) kinases; these can be activated by an even larger group of MAPK kinase kinases, which in turn are under the control of various stimuli (Raman et al., 2007). Although the specific connections between receptors primarily coupling to G_s proteins and p38 have not been elucidated, this complexity obviously allows for a single receptor to cause p38 activation by multiple pathways, making it a good candidate for differential activation in the context of LDS. ECAR is an even more distal and nonspecific cellular response. Although this is the reason that ECAR is being used as a screening tool for cellular activation, it also makes it a prime candidate for a response that can be activated by numerous pathways. Therefore, it is not surprising that different ligands acting upon β_3 -adrenergic receptors can enhance ECAR by distinct signaling pathways (Fig. 1). In general, it can be expected that the likelihood of LDS mani-

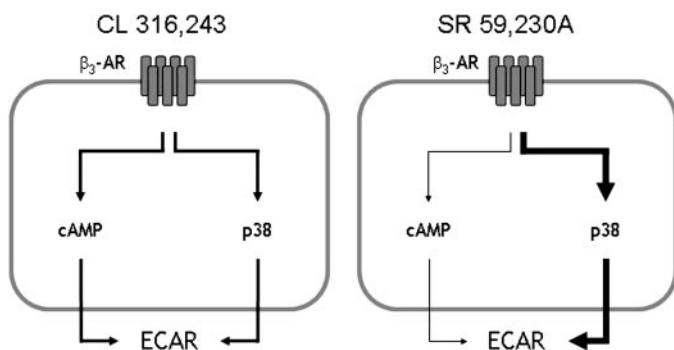


Fig. 1. Diverging and converging effects of CL316243 and SR59230A in cells expressing a low density of β_3 -adrenergic receptors (β_3 -AR) as described by Sato et al. (2007). Although both ligands are full agonists relative to isoproterenol in stimulating extracellular acidification rate (ECAR), CL316243 does so via both cAMP and p38 whereas SR59230A almost exclusively works via p38. In parallel, SR 59230A is an antagonist for cAMP formation but has greater efficacy for p38 activation than CL316243, whereas CL316243 is a full agonist for cAMP formation.

festing as activation of a single response via distinct pathways increases with the number of molecular steps between the receptor and the response. This also implies, particularly for distal cellular responses, that multiple agonists may cause the same cellular effect but nevertheless may do so by LDS (Fig. 1).

Implications for Drug Discovery

The concept of LDS in general has various implications for the process of drug discovery (Bosier and Hermans, 2007; Galandrin et al., 2007). In general, LDS allows for the possibility to obtain compounds that elicit a desired effect but cause fewer side effects, despite desired and adverse effects occurring via the same receptor. On the other hand, LDS implies that functional screening systems, particularly those for high-throughput screening, may cause numerous false positive hits or miss relevant compounds because the particular response being used for screening may not be involved in the targeted clinical effect. The example of SR59230A in the article by Sato et al. (2007) shows that compounds may appear as full or partial agonists or even antagonists, depending on the response under investigation. Moreover, despite antagonist affinity at a given receptor having been considered constant for a long time, LDS in combination with a specific functional read-out may also affect the estimated antagonist affinity (Baker and Hill, 2007). Whether these risks outweigh the potential opportunities for drug discovery remains to be determined.

The findings by Sato et al. (2007) have two additional implications for drug discovery. If a given cellular response can occur via multiple pathways that are differentially used by distinct agonists, the physiological and pathophysiological regulation of such pathways may differentially affect responses to drugs acting on the same receptor. For example, p38 is typically activated during inflammation (Abraham, 2005); hence, it can be envisioned that inflammation may affect the response to SR59230A differently than that to isoproterenol or CL316243. Another consequence of the findings by Sato et al. (2007) is that expression density may affect LDS via that receptor. Given that numerous clinical conditions have been described under which the expression density of a given receptor is up- or down-regulated (Insel et al., 2007), such receptor density-dependent LDS may also affect clinical responses in a ligand-specific manner. Perhaps even more importantly for drug discovery, the cell lines used for screening purposes typically overexpress a receptor of interest; although this may improve signal/noise ratios, it may also make it more likely to couple promiscuously and hence exhibit LDS. Taken together, LDS has the potential to explain some clinically observed differences between drugs supposedly acting on the same receptor. However, the role of LDS relative to established factors such as subtype-selectiv-

ity or pharmacokinetic properties remains to be determined across a spectrum of drugs.

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