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PERSPECTIVE

Missing Links: Mechanisms of Protean Agonism

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ABSTRACT

The concept of pharmacological efficacy has been much discussed recently with significant interest both in inverse agonists and in protean agonists (i.e., compounds with functional selectivity for different effector responses). Although first proposed in the mid-1990s, the pharmacological and therapeutic importance of these concepts is now receiving wider support. Two articles in recent issues of *Molecular Pharmacology*, Lane et al. (p. 1349, current issue) and Galandrin and Bouvier (*Mol Pharmacol* **70:**1575–1584, 2006), provide new mechanistic information on functionally selective ligands at the pharmacologically important D2 dopamine receptor and the β_1 and β_2 adrenergic receptors. Each article bridges a gap between recent biophysical studies showing distinct receptor conformations produced by different ligands and the increasing number of reports of discordant outputs by a single ligand to two effector readouts. The Lane et al. study clearly demonstrates G protein-specific actions of D_2 dopamine receptor ligands. These range from equivalent responses for $G\alpha_o$ and $G\alpha_i$ activation by norapomorphine and 7-hydroxy-2-dipropylaminotetralin to *S*-(−)-3-(3-hydroxyphenyl)-*N*-propylpiperidine, which is an agonist for $G\alpha_o$ activation and an inverse agonist at $G\alpha_{i1}$ and $G\alpha_{i2}$. Likewise, Galandrin and Bouvier describe a two-dimensional Cartesian efficacy approach in which propranolol is an agonist for extracellular signal-regulated kinase activation, probably through β-arrestin, while functioning as an inverse agonist for adenylyl cyclase activation. Thus, these two important articles further solidify the concepts of functional selectivity and protean agonism and begin to define the first postreceptor step in actions of protean agonist ligands.

The concept of receptor efficacy has been a central tenet of pharmacology since its definition (Stephenson, 1956). Efficacy describes how strongly a ligand activates a receptor, and, in contrast to intrinsic activity (Ariens, 1954), efficacy was conceived to be independent of the system used (cells, tissues, or in vivo) and the response measured (contraction, second messengers, etc.). The concept of pharmacological efficacy, however, has undergone major revisions in the last 20 years. Two key developments were: 1) the recognition of inverse agonists—compounds with "negative" efficacy that actively turn off receptors (Costa and Herz, 1989; Milligan, 2003) and 2) the recognition that efficacy cannot be expressed as a single number that determines the strength of the receptor stimulus. This occurs because receptor signals do not just activate linear pathways as previously assumed (Stephenson, 1956), but their signals can branch and engage distinct intracellular protein components. Thus, distinct outputs involving different signaling pathways may not show the same pattern of agonist dependence (Kenakin, 1995, 2001). This idea has been given many colorful (and some confusing) names, such as protean agonism, biased agonism, agonist directed trafficking of receptor stimulus, and functional selectivity. It was recently the subject of an excellent review (Urban et al., 2007).

A number of cases have been reported in which different apparent efficacies are seen for agonists acting at two effector readouts from the same receptor (Berg et al., 1998; Brink et al., 2000; Wei et al., 2003). Furthermore, recent, biophysical studies now show directly that different agonist ligands induce qualitatively different receptor conformations (Ghanouni et al., 2001; Swaminath et al., 2005; for review, see Perez and Karnik, 2005). Thus, a unidimensional efficacy term cannot account for the richness of receptor signaling. However, the mechanistic steps between distinct receptor

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ABBREVIATIONS: (*S*)-(−)-3-PPP, *S*-(−)-3-(3-hydroxyphenyl)-*N*-propylpiperidine; NPA, norapomorphine, ERK, extracellular regulated kinase; PTX, pertussis toxin.

conformations and distinct effector readouts were not directly addressed. The two articles examined here fill a gap in our understanding of this process by bridging the receptor-effector divide. One focuses on G protein selectivity and the other on distinct G protein and non-G protein mechanisms. For purposes of this article, I will use the term *protean ligand* to describe these phenomena. Although the original definition intended it to describe ligands with both agonist and inverse agonist actions at one receptor (Kenakin, 2001), it is also rather appropriate to serve as a noun for ligands that show *functional selectivity* (Urban et al., 2007).

Selective G Protein Activation. The possibility that agonists could selectively activate different G proteins was an obvious explanation for this phenomenon, but most evidence was indirect (Brink et al., 2000; MacKinnon et al., 2001). In the current issue of *Molecular Pharmacology*, an article by Lane et al. (2007) clearly establishes that mechanism. They systematically assess activation of the four primary members of the $G\alpha_i$ family ($G\alpha_{i1}$, $G\alpha_{i2}$, $G\alpha_{i3}$, and $G\alpha_o$) by different agonists at the D2L dopamine receptor. By use of the receptor-G protein fusion method and [^{35}S]guanosine 5′-*O*-(3-thio)triphosphate binding, they ensure identical expression of the associated G protein subunits and also eliminate membrane compartmentation as a reason why one G protein may be activated while another is not. Most of the D2 agonists tested can activate all four $G\alpha_i$ family G proteins. However, (*S*)-(−)-3-(3-hydroxyphenyl)-*N*-propylpiperidine [*S*-(−)-3-PPP] and *p*-tyramine are only able to activate $G\alpha_o$ and not $G\alpha_{i1}$, $G\alpha_{i2}$, or $G\alpha_{i3}$. To eliminate concerns about the artificial nature of the fusion proteins, the authors also express the $G\alpha$ subunits from a tetracycline-regulated promoter and find the same result. Furthermore, they show that high-affinity agonist binding of *S*-(−)-3-PPP, another measure of receptor-G protein coupling, also follows the same pattern with high affinity binding to the D2-$G\alpha_o$ fusion but not for $G\alpha_{i1}$, $G\alpha_{i2}$, or $G\alpha_{i3}$. Finally, *S*-(−)-3-PPP, in contrast to its activation of $G\alpha_o$, is an inverse agonist at $G\alpha_{i1}$, $G\alpha_{i2}$, and possibly $G\alpha_{i3}$. This clearly establishes *S*-(−)-3-PPP as a protean agonist at the D2L dopamine receptor and provides a molecular mechanism for differential responses in this system (Fig. 1).

Certain questions remain, however, including: 1) the tendency of most agonists reported here to activate $G\alpha_o$ better than $G\alpha_i$, 2) the complication of pertussis toxin resistance mutations in the $G\alpha$ subunits, 3) the effect of this $G\alpha$ specificity on effector responses, and 4) the ultimate in vivo functional significance of the work. Addressing these issues in reverse order, Lane et al. (2007) point out that in vivo work with *S*-(−)-3-PPP shows differential effects on pre- and postsynaptic dopamine functions (Hjorth et al., 1983). In 1983, however, one did not know about the five different dopamine receptor subtypes, so re-examination of this question with receptor-knockout models and/or improved pharmacological agents may be worthwhile. In addition, the role of $G\alpha$ selectivity in the novel pharmacology of aripiprazole (Ohta et al., 2004) will be of interest to study. Second, distinct effector mechanisms have been proposed for G_i and G_o signaling, activation of G protein-coupled inwardly rectifying potassium channel currents, and inhibition of voltage-gated Ca^{2+} channels (Sowell et al., 1997; Valenzuela et al., 1997). Thus, electrophysiological studies of these ligands in a D2-regulated neuronal system would be of significant interest. Third, the requirement for use of the pertussis toxin-insensitive mutations in the $G\alpha$ subunit carboxyl termini is a potential concern. It is noteworthy that similar results on agonist selectivity for D2L regulation of $G\alpha$ subunits was shown by Gazi et al. (2003) in Sf9 cells. They did not, however, detect inverse agonism of *S*-(−)-3-PPP at $G\alpha_{i1}$ and $G\alpha_{i2}$. That study (Gazi et al., 2003), however, does show that the general agonist selectivity seen here (Lane et al., 2007) is not due to the pertussis toxin-insensitive G proteins.

The fourth point above deserves individual scrutiny. Which of the four $G\alpha$ subunits studied here really carries out D2 receptor function in vivo? Jiang et al. (2001) show that $G\alpha_o$ is the most important $G\alpha$ subunit. In $G\alpha_o$−/− mice, dopamine-stimulated [^{35}S]guanosine 5′-*O*-(3-thio)triphosphate binding in brain and the GTP-shift for agonist binding to D2 receptors in the striatum was completely lost. In contrast, these measures of RG coupling were unaffected by knockouts of the three G_i subunits—either alone or in pairs. Thus, D2 receptors couple best to $G\alpha_o$. This was initially attributed to the greater concentration of $G\alpha_o$ versus $G\alpha_i$ subunits in the CNS. It is noteworthy that binding data in the present study (Lane et al., 2007) show that with equivalent $G\alpha$ stoichiometry, D2 receptors have a similar ability to couple to $G\alpha_i$ subunits and $G\alpha_o$ except perhaps for $G\alpha_{i2}$. D2 functional coupling, however, does show a preference for $G\alpha_o > G\alpha_{i1} \geq G\alpha_{i3} = G\alpha_{i2}$ also seen previously (Gazi et al., 2003). In particular, the pEC_{50} for the majority of agonists tested was significantly greater for the D2-$G\alpha_o$ fusion than for the D2-$G\alpha_i$ fusions. However, *n*-propyl norapomorphine (NPA) and 7-(dipropylamino)-5,6,7,8-tetrahydronaphthalen-2-ol show essentially identical EC_{50} and E_{max} values for activation of $G\alpha_o$ and $G\alpha_{i1}$ so the $G\alpha_o$ preference is agonist-dependent.

Thus Lane et al. (2007) clearly define G protein-selective agonist effects at D2 dopamine receptors (Fig. 1). They show a wide range of behaviors with NPA and 7-(dipropylamino)-5,6,7,8-tetrahydronaphthalen-2-ol having very similar abilities to activate $G\alpha_o$ and $G\alpha_{i1}$, whereas DA and quinpirole activate $G\alpha_o$ better than any $G\alpha_i$ and *p*-tyramine and *S*-(−)-

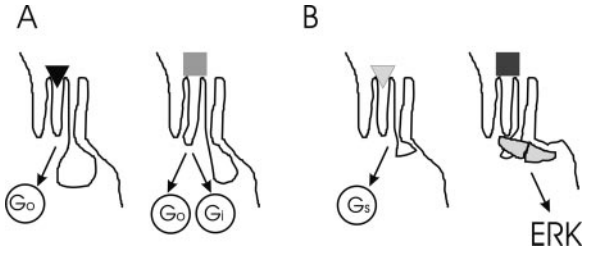

Fig. 1. Mechanisms of protean ligand action at heptahelical receptors. A, the actions of NPA and *S*-(−)-3-PPP at the D2 dopamine receptor as reported by Lane et al. (2007) are illustrated. NPA (gray square) equally activates $G\alpha_o$ and $G\alpha_{i1}$, whereas *S*-(−)-3-PPP (black triangle) activates $G\alpha_o$ (partially) and actually serves as an inverse agonist for $G\alpha_{i1}$ and $G\alpha_{i2}$. A number of other D2 ligands show intermediate behavior (including the natural agonist dopamine) in which they activate all four of the main G_i family G proteins ($G\alpha_{i1}$, $G\alpha_{i2}$, $G\alpha_{i3}$, and $G\alpha_o$) but in general, $G\alpha_o$ activation occurs with a higher potency or greater E_{max}. This range of behaviors and direct evidence of differential G protein activation provides a molecular underpinning for unique signaling properties of *S*-(−)-3-PPP in native tissues. B, protean ligand signaling at the β_1 and β_2 adrenergic receptors reported by Galadrin and Bouvier (2006) shows actions of ligands through differential actions on G protein and non-G protein pathways. At the β_1 receptor, labetalol (gray triangle) is an agonist for G_s-stimulated AC but does not activate ERK, whereas propranolol (dark gray square) is an agonist for ERK signaling but an inverse agonist for G_s-regulated AC. Here, the differential signal outputs probably use completely different mechanisms.

3-PPP are partial agonists for $G\alpha_o$ activation with virtually no activity for the $G\alpha_i$ subunits. Indeed, *S*-(−)-3-PPP even has substantial inverse agonist activity with the D2-$G\alpha_{i1}$ and D2-$G\alpha_{i2}$ fusion systems. It will be of great interest to explore the implications of this work for effector signaling, in vivo pharmacology, and therapeutics.

G Protein versus non-G Protein Mechanisms. An alternative mechanism of protean ligand action is implicated in the November 2006 issue of *Molecular Pharmacology* (Galandrin and Bouvier, 2006). Besides the classic G protein pathway that activates adenylyl cyclase, several labs have defined non-G protein signaling mechanisms through β_2 adrenergic receptor phosphorylation and recruitment of β-arrestin as a signaling scaffold that can activate extracellular regulated kinase (ERK) (Azzi et al., 2003; Shenoy et al., 2006; Werry et al., 2006). Galandrin and Bouvier (2006) examined agonist-specific signaling to adenylyl cyclase (G_s-mediated) and ERK (G_s- and β-arrestin-mediated) functional readouts. They found β_2 ligands (e.g., propranolol) that are reasonable agonists for one pathway (β-arrestin-dependent ERK signaling) and inverse agonists for the other (G_s-activated adenylyl cyclase). Although the ERK signal measured at early times is probably complicated by elements of both G_s and arrestin mechanisms (Shenoy et al., 2006), Galandrin and Bouvier (2006) provide an explicit multidimensional view of the "efficacy" of compounds, plotting the E_{max} for adenylyl cyclase signaling on the *x*-axis and the E_{max} for ERK signaling on the *y*-axis to provide a Cartesian (or vector) view of efficacy. In the case of the D2 readouts (Lane et al., 2007), that vector would have to be in four dimensions (one for each of the G proteins studied).

Thus, two key articles in *Molecular Pharmacology* push the frontier of molecular mechanisms of pathway-specific differential efficacy or protean ligand function. Both are characterized by a careful attention to quantitative analysis of drug action and each provides new but different insights into molecular mechanisms of G protein-coupled receptor action.

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