Review

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New Concepts in Dopamine D_2 Receptor Biased Signaling and Implications for Schizophrenia Therapy

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ABSTRACT

The dopamine D_2 receptor (D_2R) is a G protein–coupled receptor that is a common target for antipsychotic drugs. Antagonism of D_2R signaling in the striatum is thought to be the primary mode of action of antipsychotic drugs in alleviating psychotic symptoms. However, antipsychotic drugs are not clinically effective at reversing cortical-related symptoms, such as cognitive deficits in schizophrenia. While the exact mechanistic underpinnings of these cognitive deficits are largely unknown, deficits in cortical dopamine function likely play a contributing role. It is now recognized that similar to most G protein–coupled receptors, D_2Rs signal not only through canonical G protein pathways but also through noncanonical beta-arrestin2–dependent pathways. We review the current mechanistic bases for this dual signaling mode of D_2Rs and how these new concepts might be leveraged for therapeutic gain to target both cortical and striatal dysfunction in dopamine neurotransmission and hence have the potential to correct both positive and cognitive symptoms of schizophrenia.

Keywords: Antipsychotics, Arrestin, Dopamine, Functional selectivity, Schizophrenia, System bias

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Dopamine (DA) is a catecholamine neurotransmitter that is involved in many physiological processes in the central nervous system (CNS), and dysregulation of its function has been implicated in many CNS disorders including schizophrenia. DA mediates its effects by binding to G protein–coupled receptors (GPCRs) belonging to the D_1 or D_2 class of receptors that activate intracellular signaling cascades. Similar to most GPCRs, the classification of DA receptors was originally based on their coupling to either the stimulatory $G\alpha_s$ or the inhibitory $G\alpha_i$ G proteins (1). The D_1 class of $G\alpha_s$-coupled receptors consists of DA D_1 receptor (D_1R) and D_5R, whereas the D_2 class of $G\alpha_i$-coupled receptors includes D_2R, D_3R, and D_4R (2). G protein–mediated GPCR signaling is rapidly desensitized by the initial phosphorylation of the receptor by GPCR kinases (GRKs) followed by interaction with β-arrestins (βarrs) (3,4), which leads to inhibition of G protein signaling and subsequent internalization, dephosphorylation, and recycling of competent receptors to the plasma membrane (5). In most cellular systems, DA receptors interact with GRKs (GRK2, GRK3, GRK5, and GRK6) and βarrs (βarr1 and βarr2) (6–8) and are desensitized and internalized through this cooperative mechanism. However, over the last several years a new mode of G protein–independent GPCR signaling has emerged that is mediated via βarrs through their ability to scaffold various signaling molecules such as kinases and phosphatases (9,10). For D_1Rs and D_2Rs, it has been shown that βarr2, but not βarr1, mediates this independent signaling pathway by scaffolding signaling molecules such as extracellular signal-regulated kinase, protein kinase B (also known as AKT), and protein phosphatase 2A (11,12), which regulate certain DA-dependent physiological processes (13,14). D_2Rs are the common targets for all antipsychotic drugs (APDs), and selective D_2R-βarr2 signaling can be leveraged to discover novel therapeutic avenues in schizophrenia, which is the focus of this review.

ANTIPSYCHOTICS AND D_2R PHARMACOLOGY

DA receptor–mediated signaling has been implicated in many CNS processes, such as cognition, motor control, and reward (15–17), and dysfunctional DA receptor signaling has been implicated in many CNS disorders including schizophrenia (18–21). The DA hypothesis of schizophrenia was conceptualized from the original works of Carlsson and Lindqvist and van Rossum (22–26). Subsequently, seminal observations by groups led by Seeman and Snyder that neuroleptics or APDs bound to DA receptors (27–29) and that psychostimulants that increase brain DA exacerbated psychotic symptoms (30–33) crystallized the idea of a hyperdopaminergic state of DA in schizophrenia. Following the classification of DA receptors as D_1 and D_2, it was revealed that most APDs bound to D_2Rs, but not D_1Rs, and that blocking D_2Rs was sufficient to inhibit hyperdopaminergia (1,34,35). It was later discovered in the 1980s that clozapine, then a newer APD, had lower affinity for D_2Rs but higher affinity for the serotonin 5-HT_{2A} receptor (36). Based on the relative binding affinities for D_2 versus 5-HT_{2A} receptors, clinical APDs were either termed “typical” or “first-generation” APDs (haloperidol and chlorpromazine) or

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"atypical" or "second-generation" APDs (clozapine, risperidone, and olanzapine). However, for both types of APDs, binding to the D_2R is a common property, and it was shown that these APDs mediated their actions predominantly by acting as antagonists or inverse agonists at D_2Rs (37,38). Although both types of APDs are clinically effective, there are significant differences in their therapeutic and side-effect profiles. Schizophrenia is characterized by positive (hallucinations, delusions), negative (alogia, anhedonia, avolition), and cognitive symptoms. The typical APDs are effective at targeting the positive symptoms of schizophrenia but have several motor-related side effects called extrapyramidal symptoms (EPS). Although EPS induced by typical APDs are a result of excessive D_2R binding in striatal regions, it is thought that the therapeutic effectiveness also requires striatal D_2R binding with presumably faster dissociation rates (20). Atypical APDs have overcome some of the problems with typical APDs in the clinic and are relatively better at targeting the symptoms of schizophrenia without inducing EPS. However, the atypical APDs have their own distinct side-effect profile, which includes weight gain, agranulocytosis, and hypotension. None of the APDs efficiently target the cognitive dysfunction observed in schizophrenia, which precedes the positive and negative symptoms.

Several pieces of evidence argued against hyperdopaminergia as the sole biochemical manifestation in schizophrenia (39), and evidence from Pycock *et al.* (40) and Weinberger (41) led to a revision of the DA hypothesis to include cortical hypodopaminergia in addition to subcortical hyperdopaminergia. Based on this updated DA hypothesis, it was understood that all APDs that are essentially D_2R blockers would reverse only subcortical hyperdopaminergia but not cortical hypodopaminergia. To target these opposite phenomena simultaneously in a pharmacological manner, one would have to devise an APD that is an antagonist and an agonist at the same time. To achieve this, partial agonism was the novel approach used, as pharmacologically partial agonists are also antagonists as they block binding of the endogenous ligand. Aripiprazole, synthesized by Otsuka Pharmaceutical (Tokyo, Japan), is a partial agonist at D_2Rs but retains most of the properties of other atypical APDs, such as 5-HT_{2A} receptor binding. Aripiprazole was termed a "third-generation" APD and has been shown to be effective in the clinic with a lower risk of EPS and metabolic side effects. However, even aripiprazole with its partial agonist activity has in large measure failed to reverse the cognitive deficits in schizophrenia. Therefore, after 60 years of development in APD therapeutics, these drugs are only partially effective and lack complete efficacy toward all symptoms of schizophrenia. New concepts in GPCR signaling provide a compelling strategy for overcoming these unmet needs in the pharmaceutical intervention of schizophrenia.

The discovery that D_2Rs can signal not only through G protein pathways but also through the ability of βarr2 to scaffold distinct signaling complexes has revealed novel avenues for pharmacologically targeting D_2Rs for APD therapy. The initial observations that GPCRs, like the β_2-adrenergic and the mu opiate receptors, had the ability to signal both in vitro and in vivo through βarrs paved the way to explore this concept for other GPCRs (9,42,43). Our group provided the first evidence that DA-dependent behaviors could also be mediated via a βarr2-dependent manner at the D_2R (11) through the ability of βarr2 to scaffold protein phosphatase 2A, protein kinase B, and glycogen synthase kinase 3β in the mouse striatum. Genetic inactivation of βarr2 (βarr2 knockout) resulted in disruption of this signaling complex and a reduction in DA-dependent locomotor behavior (11,44), which essentially mimics the actions of APDs. In in vitro assays, all clinically effective APDs block D_2R-βarr2 recruitment (45), which is consistent with our previous findings in βarr2 knockout mice. It has also been shown that G protein–mediated signaling via dopamine- and cyclic adenosine monophosphate (cAMP)-regulated phosphoprotein, Mr 32 kDa (DARPP-32), but not βarr2 signaling via glycogen synthase kinase 3β, exacerbates the manifestation of haloperidol-induced catalepsy (14,46). For the few GPCR signaling systems in which G protein versus βarr pathways have been critically examined, invariably these two signaling modes mediate distinct cellular and behavioral paradigms (47–49). Therefore, these data suggested that selectively targeting the D_2R-βarr2 pathway and leveraging this dual signaling concept of GPCRs called functional selectivity or biased signaling might be beneficial in APD development and might present added benefits as is discussed below.

MOLECULAR AND MECHANISTIC BASES FOR FUNCTIONAL SELECTIVITY/BIASED SIGNALING AT D_2R

Functional Selectivity

The concept of functional selectivity or biased signaling for GPCRs originally arose more than 20 years ago from cellular observations of distinct pharmacological profiles of ligands or the ability of certain GPCRs to engage multiple G proteins (50–52). This concept has now been broadened to account for the realization that GPCRs have the ability to mediate signaling not only through activation of G proteins but also through the ability of βarrs to scaffold distinct signaling events (48). We now recognize that the ability of a ligand to act as agonist, partial agonist, or antagonist depends not only on its chemical structure (53) but also on the conformation of receptor itself (54) and perhaps more importantly the complement of intracellular signal transducer proteins such as G proteins, GRKs, and βarrs expressed in a particular cell type (Figure 1A) (42,55,56). Considering that the majority GPCRs are activated in vivo by a single endogenous ligand and naturally occurring GPCR mutants that are functionally selective for either of these pathways are rare, it is likely that most in vivo manifestations of GPCR functional selectivity rely on the cellular complement of signal transducers. We briefly review below how each of these three aspects of functional selectivity have been addressed for the D_2R and how they might impact therapeutic approaches for treatment of schizophrenia.

Ligand-Induced Functional Selectivity at D_2Rs

DA is the endogenous ligand for D_2R, which is equally effective at activating both $G\alpha_i$-mediated cAMP inhibition and βarr2 recruitment and can regulate DA-dependent physiological processes. To decipher the actions of DA on each D_2R pathway, functionally selective ligands might prove