REVIEW

CNS Neuroscience & Therapeutics

Dopamine Receptor Homooligomers and Heterooligomers in Schizophrenia

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Keywords
Dimers; Dopamine receptors; Heteromers; Homomers; Schizophrenia.

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doi: 10.1111/j.1755-5949.2010.00228.x

SUMMARY

Over the past two decades the dopamine D2 receptor has been undoubtedly the most widely studied dopamine receptor for the therapeutic treatment of schizophrenia, as the majority of antipsychotics exhibit antagonism at this receptor. However, the cognitive symptoms of the disorder are mostly resistant to the majority of available antipsychotic treatments and, as a result, there is a critical need to develop novel therapies that ameliorate all symptoms. The recognition that dopamine receptors, such as all G protein-coupled receptors (GPCRs), exist as oligomeric complexes has provided new avenues for drug design in the search for novel therapies. Furthermore, that it is now known that dopamine receptors can form heteromers, such as the dopamine D1–D2 receptor heteromer, with pharmacology and function distinct from its constituent receptors, has significantly expanded the range of potential drug targets. The aim of this review is to discuss the therapeutic relevance of these dopamine receptor oligomers to schizophrenia and to address the potential value of dopamine receptor heteromers in the search for new therapeutic strategies.

Introduction

Dopamine receptors, members of the G-protein coupled receptor (GPCR) family, consist of five receptor subtypes that are divided into two major subclasses: the D1-like (D1, D5) and D2-like (D2, D3, D4) receptors, that are typically coupled to the stimulatory Gs and inhibitory Gi proteins, respectively. Although classical thinking has depicted GPCRs as monomeric entities, and has modeled receptor ligand binding and signal transduction properties after this idea, numerous reports have now shown that GPCRs exist as dimers and higher order oligomeric complexes [1–3], a characteristic that is now accepted as a general feature of GPCR biology. Indeed, studies assessing the functional relevance of oligomerization have now shown that it plays a significant role in important cellular processes. Specifically, for some receptors it appears oligomerization is not only critical for receptor transport and proper plasma membrane expression [4–9], but also may be integral in regulating the magnitude of the physiological response induced by receptor activation through the process of cooperative binding [10–12]. In addition, the discovery that GPCRs such as dopamine receptors could form heteromeric complexes [13–23] has opened up novel avenues of research for drug discovery, as many of these receptor heteromers exhibit pharmacological and functional characteristics distinct from their constituent receptors. The dopamine D1–D2 receptor heteromer, for example, first identified in rat and human striatum in 2004 [20], was shown to couple to the Gq protein, a finding that effectively linked dopamine to calcium signaling in brain [24].

The pharmacological modification of dopamine transmission has long been employed as a therapeutic tool in the treatment of many dopamine-related disorders, with the D2 receptor being considered to be the most clinically relevant to schizophrenia. It is noteworthy, however, that although almost all antipsychotics exhibit antagonistic properties at this receptor, a variety of neurochemical, pharmacological, and neuroimaging evidence has emerged implicating other dopamine receptor oligomers, both homomeric and heteromeric, as being potentially important therapeutic targets. This chapter will review the recent advances that have contributed to the understanding of how these receptor oligomers may be important to the therapeutic management of schizophrenia, with specific emphasis on dopamine receptor heteromers. Because of lack of evidence supporting a role for the D4 and D5 receptor oligomers in this disorder, however, these receptors will not be discussed.

The Dopamine Hypothesis of Schizophrenia

Schizophrenia is a chronic mental illness commonly characterized by a combination of positive (i.e., psychoses) and negative (i.e., apathy) symptoms as well as a broad range of cognitive deficits (i.e.,

poor learning, memory function). The classical dopamine hypothesis of schizophrenia, which posited subcortical dopaminergic hyperactivity as the primary disturbance contributing to the pathophysiology of the disorder, was derived from studies that showed that the symptomatic treatment of schizophrenia by antipsychotics was based on their ability to antagonize the dopamine D2 receptor [25,26] and, conversely, that dopamine receptor agonists could induce or enhance positive symptoms [27,28]. Furthermore, recent advances in neuroimaging assessing dopamine neurotransmission have lent credence to this hypothesis showing that schizophrenia was associated with presynaptic striatal dopamine dysfunction consisting of exaggerated striatal dopamine storage and/or synthesis [29–31] and enhanced basal or stimulated dopamine release [32–35].

However, despite the success of antipsychotics in relieving the positive symptoms of schizophrenia, these drugs were not efficacious at relieving the negative and cognitive impairments of the illness. This suggested that the classical dopamine hypothesis of schizophrenia was incomplete and it was hypothesized almost 20 years ago that, given the known circuitry linking subcortical and cortical brain structures, the mesolimbic hyperdopaminergia observed in schizophrenia may coexist with low prefrontal cortical dopamine activity [36]. At about the same time, the critical role of prefrontal cortex (PFC) dopamine D1 receptors in the regulation of working memory and cognitive functioning was becoming apparent [37,38], indicating a possible link between reduced D1 receptor activity in PFC and the cognitive impairments in schizophrenia. Indeed, it was shown shortly thereafter that haloperidol or clozapine administration in nonhuman primates led to a downregulation of PFC D1 receptors [39] but only clozapine, an atypical antipsychotic that preferentially induced dopamine release from PFC [40–42], was effective in ameliorating both the positive, negative, and cognitive symptoms of schizophrenia [43,44]. Furthermore, clinical imaging studies have also since revealed that there is an absence of normal PFC activation during working memory performance in schizophrenic patients [45–48], further verifying the importance of this brain region in the therapeutic management of cognitive deficits. Thus, based on these findings the classical hyperdopaminergic hypothesis of schizophrenia has now evolved to include a deficit in prefrontal cortical dopamine functioning [49,50], which together significantly contribute to the pathophysiology of schizophrenia.

Dopamine Receptor Homomers

Antipsychotic therapy has provided an immeasurable therapeutic benefit for the treatment of the positive symptoms of schizophrenia, however these drugs exhibit poor efficacy in alleviating both the negative symptoms and cognitive deficits of the disorder. As a result of the potential importance of PFC D1 receptors in ameliorating cognitive impairments in schizophrenia, it is logical that research into the efficacies of D1 receptor agonists as therapeutic agents have been proposed [50,51]. However, a lack of suitable agonists has made this difficult, in part due to the short half-lives of these drugs, and additionally due to the development of drug tolerance resulting from D1 receptor internalization. One potential candidate, dihydrexidine, has high affinity and potency at the D1 receptor but is not selective, with only approximately a 10-fold higher affinity for the D1 receptor than the D2 receptor [52]. Nonetheless, this drug appears to be well tolerated in patients when administered acutely [53] and to significantly increase prefrontal brain activity in schizophrenia [54].

Dopamine D2 receptors have been the most widely studied therapeutic target for schizophrenia as antipsychotics have almost universally exhibited antagonism at this receptor. However, studies are now beginning to emerge suggesting that partial agonism may be a suitable therapeutic strategy, especially given the combined striatal hyperdopaminergia and prefrontal hypodopaminergia that is characteristic of schizophrenia. In essence, as a partial agonist would exhibit lower intrinsic activity at the D2 receptor than dopamine, these drugs could presumably act as functional antagonists under conditions of elevated dopamine and, conversely, enhance dopamine receptor signaling under conditions of dopamine depletion [55].

At present there are few antipsychotic drugs that can be characterized as partial agonists at the D2 receptor and the ones that do exist also exhibit agonist and/or antagonist properties at other receptor complexes. Nonetheless, the therapeutic benefits of these drugs appear promising, indicating that these types of antipsychotics may warrant additional attention. For example, preclinical studies have shown that the putative antipsychotic WS500-30, a partial D2 receptor agonist and serotonin transporter blocker, may have potential for the therapeutic management of both psychosis and cognitive defects in schizophrenia [56]. Similarly, the mGlu2/3 receptor glutamate agonist LY404039, also with partial activity at the D2 receptor [57–59], is efficacious in alleviating both the positive and negative symptoms [60]. Another drug with apparent partial D2 receptor agonism is the antipsychotic aripiprazole [61]. This drug, which is also a serotonin 5-HT_{1A} receptor partial agonist, and antagonist at 5-HT_{2A} receptors, also exhibits therapeutic benefits for the positive and negative symptoms [62,63] and has also recently been reported to have significant cognitive benefits [64]. However, it is noteworthy that aripiprazole also appears to exhibit functional selectivity, as *in vitro* studies have shown large variations in its intrinsic activity and potency at the D2 receptor that were cell line-dependent [61,65–67]. These findings suggest that the effects of aripiprazole may extend beyond simple partial agonism, although further investigation is required to clarify the therapeutic relevance of the functional selectivity of this antipsychotic *in vivo*.

There has been considerable debate as to the value of the D3 receptor as a therapeutic target. Upon the discovery of the D3 receptor 20 years ago, it was noted that this receptor was a molecular target for drugs used for the treatment of psychiatric symptoms [68] and studies assessing the pharmacology of the D3 receptor *in vitro* have since shown that this receptor shows affinity for many antipsychotic drugs [69]. However, although many of these drugs may have exhibited affinity for the D3 receptor, it was only the D2 receptor that showed significant drug occupancy at therapeutic doses [70–72]. For example, in a recent neuroimaging study it was reported in schizophrenia patients that while therapeutic doses of antipsychotics did block D2 receptors, they did not block D3 receptors as estimated by the D3 receptor preferring radiotracer

[11C]-(+)-PHNO [72]. An elevation in the [11C]-(+)-PHNO signal in the D3 receptor rich globus pallidus was observed however, a finding that was potentially indicative of increased D3 receptor availability or affinity with antipsychotic use. As D3 receptors exhibit a preferential limbic distribution [68], and high sensitivity to background dopamine levels [73], the authors proposed that small changes in D3 receptor expression may be functionally significant in neuropsychiatric disorders. However, this group was unable to find any brain region-specific differences in displaceable D3 receptor binding using [11C]-(+)-PHNO in drug-free schizophrenia patients [74], suggesting that this receptor may have little significance in the etiology of the disease.

Dopamine Receptor Heteromers

Dopamine receptors exist as receptor homomers and can additionally form heteromeric receptor complexes that can exhibit pharmacologies and functional properties distinct from their constituent receptors, and although these receptor complexes represent a relatively new area of neuroscience research, their value as potentially important therapeutic targets for the treatment of neuropsychiatric disorders, including schizophrenia, is quickly becoming apparent.

The Dopamine D1–D2 Receptor Heteromer

Heteromerization of the D1 and D2 receptor was initially shown by coimmuno precipitation from rat and human striatum [20], findings that were soon thereafter confirmed by fluorescence resonance energy transfer (FRET) studies [75–77], now a common tool used for the identification of receptor oligomers. Although first performed in cells [75,76], quantitative FRET *in situ* has now been utilized to verify the presence of D1–D2 receptor heteromers both in neonatal cultured rat striatal neurons and in adult rat striatum [77], and several lines of evidence now suggest this receptor heteromer may have etiological significance in schizophrenia.

Although the dopamine hypothesis of schizophrenia postulates hyperactivity of subcortical dopamine transmission, it has also been proposed that abnormal regulation of calcium signaling may constitute the central dysfunction that is responsible for generating the psychopathology of schizophrenia [78]. As neither of the most abundant dopamine receptors (D1 or D2) directly regulated calcium signaling, it was difficult to reconcile these two streams of evidence. However, the unification of these mechanistic hypotheses occurred when it was reported that coactivation of both receptors within the dopamine D1–D2 receptor heteromer led to a novel Gq-linked increase in intracellular calcium that was distinct from its constituent receptors [20,24] and the D1–D2 heteromer also exhibited unique cell surface localization, internalization, and transactivation properties [75]. Furthermore, D1–D2 heteromer-mediated signaling could be attenuated by the D2 receptor antagonist raclopride [24,77], a finding suggestive of the D1–D2 heteromer as being a pharmacological target for neuroleptics *in vivo*. Indeed, it was recently demonstrated that the antipsychotic clozapine could uncouple the subset of D1–D2 receptor heteromers that were in their high affinity state [79,80]. As heteromerization of D1 and D2 receptors becomes enhanced upon coactivation of the receptors [76], and thus would presumably be elevated in schizophrenia, it was suggested that the therapeutic effects of atypical antipsychotics may result, at least in part, from D1–D2 heteromer dissociation [76].

Further evidence in support of a role for the D1–D2 heteromer in schizophrenia came from studies assessing the functional role of the D1–D2 heteromer using the selective agonist SKF 83959 [24]. In these studies, the acute or short-term administration of SKF 83959, but not the D1 homomer-selective agonist SKF 83822, increased the expression of brain-derived neurotrophic factor (BDNF) in striatum [77], a neurotrophin that has been repeatedly linked to schizophrenia [81–85]. It is likely that the ability of the D1–D2 heteromer to induce BDNF expression came from its ability to activate striatal calcium calmodulin kinase IIα (CaMKIIα) [24,86], a transcription factor that is involved in the epigenetic regulation of BDNF [87]. Interestingly, CaMKIIα has also been recently linked to schizophrenia as CaMKIIα heterozygous knock-out mice display features analogous to an animal model of schizophrenia [88]. Specifically, in addition to their exhibiting enhanced activity-dependent dopamine release during repeated presynaptic stimulation [89], they show markedly upregulated levels of the agonist-induced high affinity state of D2 receptors in striatum, a characteristic that has been noted as being a consistent marker for animal models of psychosis [88,90–92].

From a neuroanatomical perspective it is perhaps noteworthy to mention that while the D1 receptor is largely segregated to the direct striatonigral pathway, and the D2 receptor is predominantly localized to the indirect striatopallidal pathway [93,94], there also exists a physiologically relevant fraction of striatal neurons that express both D1 and D2 receptors [20,95–98]. For the most part research into the functional importance of these neurons has been ignored, most likely as a result of methodological difficulties attempting to isolate them. However, these coexpressing neurons may potentially represent a third, currently unrecognized neuronal pathway that may also have some measure of control over thalamic output. Furthermore, the presence of the D1–D2 heteromer in these neurons implicates them as being significant to dopamine transmission and schizophrenia. Although pharmacological isolation of the D1–D2 heteromer has previously been unattainable, as most dopamine agonists activate the D1–D2 heteromer in addition to the D1 and D2 homomers, the recent identification of SKF 83959 as being D1–D2 heteromer-specific [24] may provide the needed stepping stone to elucidate the importance not only of the D1–D2 heteromer in schizophrenia, but also assist in increasing our understanding of the circuitry and physiological relevance of D1 and D2 receptor coexpressing neurons. That SKF 83959 sits within a unique binding pocket of the D1–D2 heteromer [24] also gives hope for the eventual development of D1–D2 heteromer-specific antagonists, drugs that could be potentially beneficial as therapeutic agents in schizophrenia.

The $A2_A$-D2 Receptor Heteromer

Another heteromic complex with implications for schizophrenia is one formed by the adenosine $A2_A$ receptor and the D2 receptor. The $A2_A$-D2 receptor heteromer, first identified by coimmunoprecipitation studies in SH-SY5Y neuroblastoma cells [13], has since

been shown to exist in living cells using FRET and BRET [99,100], and studies now suggest it likely that the $A2_A$-D2 heteromer exists along the ventral striatopallidal pathway [101,102], an important target pathway for antipsychotic drugs. Consistent with reports showing reduced striatal D2-like agonist binding and an attenuation of D2 receptor-mediated effects on adenylyl cyclase upon $A2_A$ receptor activation [103–105], it has been suggested that one role of the $A2_A$ receptor within the $A2_A$-D2 heteromer might be to dampen D2 receptor signaling [102,106]. This line of reasoning also concurred with evidence of antipsychotic effects of the $A2_A$ receptor agonist CGS 21680 in animal models of schizophrenia [107,108]. Based on these findings it was postulated that the etiology of schizophrenia may include a D2 receptor-induced reduction in $A2_A$ activity or an interruption of normal $A2_A$-D2 receptor interactions and therefore $A2_A$ receptor agonists, along with low-dose D2 receptor antagonists, may represent a potential therapeutic treatment for schizophrenia by reducing the proportion of D2 receptors in the agonist-induced high affinity state and reducing receptor signaling [106,109].

Other Dopamine Receptor Heteromers

Similar to the $A2_A$-D2 receptor heteromer, the higher order heteromer $A2_A$-D2-mGlu5, shown to exist in living cells using bimolecular fluorescence complementation [110], has also been suggested as a therapeutic target for schizophrenia by counteracting exaggerated D2 receptor signaling in the ventral striatopallidal pathway [109]. In addition, it has been shown by FRET that $A2_A$ receptors can also form heteromers with dopamine D3 receptors. This $A2_A$-D3 heteromer exhibits a similar antagonistic interaction on adenylyl cyclase activity as observed in the $A2_A$-D2 receptor heteromer [16], although the therapeutic relevance of this heteromer to schizophrenia remains unexplored.

Coimmunoprecipitation, FRET, and/or BRET techniques have also shown the existence of the D2–D3 heteromer [21], the D2–D5 heteromer [15] and the D1–D3 heteromer [14,111]. As a result of the relative novelty of these receptor complexes, little is yet known regarding their potential relevance to schizophrenia. However, a putative role for the D2–D3 heteromer as a target for antipsychotics, and in particular antipsychotics with partial D2/D3 receptor agonism, has been suggested [112]. Furthermore, the D1–D5 heteromer has been hypothesized to be involved in acetylcholine release [113], a neurotransmitter that has been connected to the regulation of dopamine transmission [114]. It has also been shown that activation of the D3 receptor elevates D1 receptor-mediated responses in neurons that coexpress both receptors [14,111], a finding that may be of relevance to the improvement of negative symptoms and/or cognitive deficits in schizophrenia.

Concluding Remarks

At present, the dopamine D2 receptor remains the predominant target for the therapeutic management of the positive symptoms of schizophrenia, however given the dual nature of schizophrenia, that is, subcortical hyperdopaminergia and cortical hypodopaminergia, the alleviation of all the positive, negative, and cognitive deficits with a single drug remains difficult to achieve. Ideally, dopamine drugs that exhibit antagonism at D2 receptors and are agonists at D1 receptors would be an efficacious treatment and indeed, preclinical studies have shown that the drug stepholidine may eventually represent such a treatment [115–117]. However, given the oligomeric nature of dopamine receptors, the development of dimeric or multimeric ligands also represents a promising therapeutic strategy as these ligands could interact simultaneously with more than one receptor and potentially increase drug potency. A significant advantage of this approach can be clearly seen for receptor heteromers as bivalent drugs for heteromers could potentially be generated by linking two monovalent drugs [118]. Because all neuroleptics would also invariably target dopamine receptor heteromers containing the D2 receptor, such as the dopamine D1–D2 receptor heteromer, these bivalent drugs would also help clarify whether the ameliorating effects of antipsychotics are the result of binding to D2 receptor homomers, D2 receptor-containing heteromers or both. As heteromerization of dopamine receptors adds a new level of diversity to dopamine receptor structure, pharmacology and function new approaches to therapeutic drug design and discovery must be taken and the development of pharmacological agents selective for these receptor heteromers would undoubtedly be beneficial for the future therapeutic management of schizophrenia.

Acknowledgments

This work was supported by a grant from the National Institute on Drug Abuse (to S.R.G. and B.F.O.), and an Ontario Mental Health Foundation Postdoctoral Fellowship (to M.L.P.). S.R.G. holds a Canada Research Chair in Molecular Neuroscience.

Conflict of Interest

The authors have no conflict of interest.

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