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Novel dimensions of D_3 receptor function: Focus on heterodimerisation, transactivation and allosteric modulation ☆

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Abstract
The brain's complexity derives not only from the way the intricate network of neurons is wired, but also by protein complexes that recognize and decode chemical information. G protein-coupled receptors (GPCRs) represent the most abundant family of proteins mediating neurotransmission in the brain, and their ability to form homo- and heteromers which amplifies the scope for synaptic communication and fine-tuning. Dopamine receptors are important drug targets and members of both the D_1/D_5 and $D_2/D_3/D_4$ receptor families form homo- and heteromers. The present article focuses on D_3 receptor homo- and heteromers, in particular, those formed in association with their D_2 counterparts. We highlight the binding profiles and mechanisms of interaction with D_3-D_3 homomers and D_3-D_2 heteromers of: first, the PET ligand and potent agonist [^{11}C]-(+)-PHNO; second, the novel, bitopic/allosteric dopamine D_3 receptor antagonist, SB269,652; and third, diverse partial agonists like antipsychotic and aripiprazole. Molecular mechanisms of interplay between the two protomers of heteromeric D_3-D_2 complexes are likewise discussed: for example, "transactivation", whereby recruitment of one member of a heteromer harnesses signalling pathways is normally coupled to the other protomer. Finally, D_1 receptor heteromers are also taken into consideration in deciphering the nature of interfaces required to stabilize dimeric assemblies and permit their interaction with G proteins. Improved understanding of D_3 as well as D_2 and D_1 receptor complexes should yield important

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insights into their physiological roles and pathological significance, and permit the development of novel drug classes with potentially distinctive functional profiles and improved therapeutic windows.

1. Introduction

G protein-coupled dopamine receptors (D_1, D_2, D_3, D_4, D_5) mediate the physiological functions of the neurotransmitter dopamine, including voluntary movement, motivation, affect, learning, reward and hormonal regulation. Dysregulation of dopaminergic neurotransmission is implicated in several neurological and psychiatric disorders, such as Parkinson's disease, Huntington's disease, attention deficit hyperactivity disorder, Tourette's syndrome, schizophrenia, bipolar disorder and drug abuse. Pharmacological agents targeting dopamine receptors, mostly D_2 and D_3 receptors, are clinically relevant as antiparkinson drugs and antipsychotic.

Classically, dopamine receptors are divided into two superfamilies that differ in their brain distribution, binding profiles and functional properties. D_1-like receptors (D_1 and D_5) are principally coupled to the stimulatory Gα-proteins and enhance the activity of adenylyl cyclase (AC), whereas D_2-like receptors (D_2, D_3, and D_4) are primarily coupled to the inhibitory Gi-proteins and suppress the activity of AC. Alternate splicing of D_2 receptor mRNA leads to the generation of two D_2 receptor isoforms: D_2 short (D_{2S}) and D_2 long (D_{2L}), which have been associated (though not exclusively) with presynaptic and postsynaptic populations of D_2 receptors, respectively. Recent research has revealed many novel facets of a dopamine receptor function. In addition to their primary activity on cAMP signaling, dopamine receptors recruit G protein-independent mechanisms *via* interactions with other proteins, such as β-arrestins.

The general assumption that a receptor is engaged with its agonists in exclusively one type of signaling event has succumbed to a pluridimensional model of different active states of the receptor with multiple downstream signaling pathways. Amongst various mechanisms underlying receptor "versatility" and/or "multidimensionality", oligomerization has received general recognition as a mechanism for fine-tuning, diversifying and amplifying receptor signalling (Maggio et al., 2007, 2009; Ferré et al., 2014). Indeed, the three most representative dopamine receptors subtypes, D_1, D_2 and D_3, have all been shown to form heteromeric complexes with properties distinct from those of their monomeric counterparts (Scarselli et al., 2001; Lee et al., 2004; Fiorentini et al., 2008). D_2 and D_3 receptors display a high degree of sequence similarity, they share a predicted binding site for dopamine and also show similar patterns of signal transduction, though D_3 receptors appear to couple less robustly to intracellular messengers like AC. While the neurobiology and relevance of D_2 sites is well established, the significance of D_3 receptors still remains under investigation. The density of dopamine D_3 receptor is generally lower and its distribution in rodent and human brain is more restricted than that of D_2 receptors (Diaz et al., 2000). Nonetheless, they are functional and well represented in brain areas like the ventral striatum (*nucleus accumbens*), thalamus, cortex and cerebellum. Presynaptically, the D_3 receptor is colocalized with D_2 sites on mesolimbic and nigrostriatal dopaminergic neurons originating in the ventrotegmental area and *substantia nigra pars compacta*, respectively (Diaz et al., 2000). Considering their strategic location in the limbic system, D_3 receptor ligands have received considerable interest as therapeutic targets for the treatment of the positive symptoms of schizophrenia, while blockade of D_3 receptors in frontal cortex may alleviate the neurocognitive and social cognitive deficits of the disorders (Millan and Brocco, 2008). In addition, D_3 autoreceptors mediate neuroprotective and neuroplastic actions on dopaminergic neurons, while postsynaptic D_3 receptors modulate the antiparkinson properties of agents acting *via* D_2 receptors (Joyce and Millan, 2007; Millan et al., 2004a).

Given the relevance of D_3 and D_2 receptors to the etiology and treatment of many CNS disorders, this review focuses on the pharmacological properties and therapeutic significance of dopamine D_3 receptor heteromerization, especially with their D_2 receptor counterparts.

2. D3 dopamine homo- and heteromers

G protein-coupled receptor (GPCR) homo- and heteromerization has been recognized to be of high relevance to the molecular properties of GPCRs (Maggio et al., 2007, 2009; Ferré et al., 2014). Although GPCR heteromerization between class C GPCRs such as the GABAB receptor is considered obligatory (Jones et al., 1998), the role of oligomerization between class A GPCRs, such as dopamine D_3 receptor, is a subject of intense debate and has been the focus of an extensive number of experimental investigations. In fact, some members of class A GPCRs, even if they are capable of activating G proteins and β-arrestin effectively as monomers (Whorton et al., 2007) form dimers and oligomers raising the question of their functional and pharmacological significance.

Since the study of Nimchinsky et al., 1997 shows that dopamine D_3 receptors are expressed in the brain as dimers and tetramers, others have confirmed that D_2 and D_3 receptors exist as homomers and heteromers (Scarselli et al., 2001; Pou et al., 2012). Interestingly, D_3 receptor activity and expression may be regulated through the expression of an alternatively spliced, truncated receptor isoform termed 'D_3nf'. The D_3nf truncated isoform is inactive *per se* but it can be rescued by the tail part of the D_3 receptor (D_3tail) suggesting a possible swapping domain mechanism in the formation of D_3/D_3nf heteromers (Scarselli et al., 2003).

As regards native, non-mutated forms of dopaminergic receptor, several studies have examined the mechanism of dopamine receptor dimerization. Using a functional complementation assay allowing for control of the two monomeric