



Review

Functional Selectivity of Dopamine D₁ Receptor Signaling: Retrospect and Prospect

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Abstract: Research progress on dopamine D₁ receptors indicates that signaling no longer is limited to G protein-dependent cyclic adenosine monophosphate phosphorylation but also includes G protein-independent β -arrestin-related mitogen-activated protein kinase activation, regulation of ion channels, phospholipase C activation, and possibly more. This review summarizes recent studies revealing the complexity of D₁ signaling and its clinical implications, and suggests functional selectivity as a promising strategy for drug discovery to magnify the merit of D₁ signaling. Functional selectivity/biased receptor signaling has become a major research front because of its potential to improve therapeutics through precise targeting. Retrospective pharmacological review indicated that many D₁ ligands have some degree of mild functional selectivity, and novel compounds with extreme bias at D₁ signaling were reported recently. Behavioral and neurophysiological studies inspired new methods to investigate functional selectivity and gave insight into the biased signaling of several drugs. Results from recent clinical trials also supported D₁ functional selectivity signaling as a promising strategy for discovery and development of better therapeutics.

Keywords: functional selectivity; dopamine D₁ receptor; clinical implications



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1. A Brief Introduction of Functional Selectivity

The term functional selectivity [1] was first introduced in 1994 and soon thereafter other reports referred to this phenomenon alternately as “agonist trafficking of signaling”, “differential engagement”, or “biased agonism”. The concept of functional selectivity essentially means that a ligand may have different actions at two or more signaling pathways mediated by the same receptor. Ligand actions may range from full agonism at one pathway and antagonism at another. Before functional selectivity was introduced, a ligand was defined as either an agonist or antagonist for one canonical signaling pathway mediated by the target receptor. This simplified notion had to be abandoned once it became clear that multiple signaling pathways could be involved by the activation of a receptor. This pharmacological insight contributed significantly to the development of the concept of functional selectivity. Meanwhile, functional selectivity also benefited from the progress on structural biology where the discovery of heterodimers and some detailed crystal structures inspired understanding of additional signaling properties of a receptor. The concept of functional selectivity has evolved. Initially, functional selectivity was studied using a “paired model”, such that a newly discovered receptor signaling pathway was compared with the traditional canonical pathway. Now, the “network model” is more appreciated whereby all signaling pathways related to a receptor are treated equally and the signaling complex as a whole accounts for the pharmacological property of a ligand. Functional selectivity has become a major pharmacological research front and more relevant as additional signaling pathways were discovered. The full signaling properties of many new and some “old” ligands have been studied or reinvestigated to better understand their engagement of targets in more precise ways and potentially to improve their therapeutic index. To grasp the significance of functional selectivity of dopamine D₁ ligands, it is important first to

review all canonical and novel signaling pathways related to dopamine D₁-like receptors (D₁Rs).

2. G Protein-Dependent Cyclic Adenosine Monophosphate (cAMP) Phosphorylation

Dopamine receptors belong to the G-protein-coupled receptor (GPCR) group of receptors and classically have been linked to adenylyl cyclase. D₁Rs interact with G $\alpha_{s/olf}$ to stimulate adenylyl cyclase and produce cAMP (Figure 1). Nobel Laureate Paul Greengard and his colleagues made a series of discoveries in the 1970s [2] that revealed that dopamine interacts with receptors to cause an increase in cAMP, activates protein kinase A (PKA), and in turn phosphorylates other proteins. Therefore, G protein coupled adenylyl cyclase activation traditionally was used as the canonical signaling pathway for D₁R.

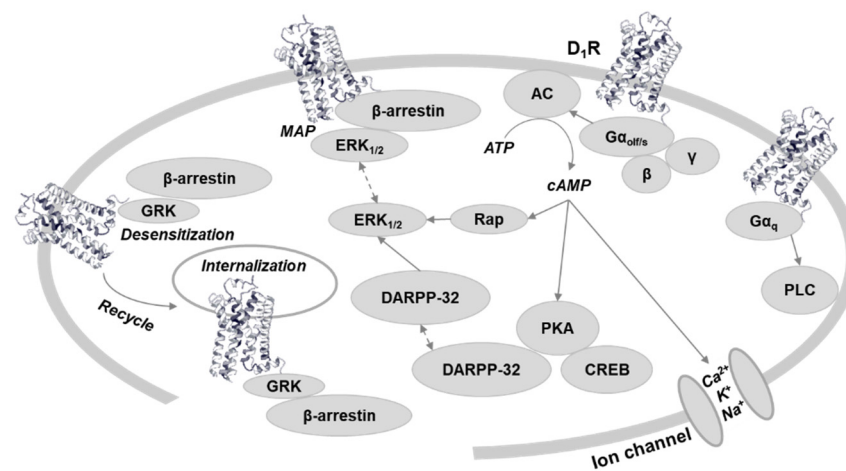


Figure 1. Dopamine D₁ receptor-related signaling. The traditionally canonical G protein coupled cAMP signaling potentially could be subdivided based on G protein subtype [3] and PKA subunit [4]. G protein independent, β -arrestin-related signaling acts through MAP kinase phosphorylation [5], and has cross talk with cAMP signaling [6,7]. Receptor recycling is also regulated by β -arrestin. Regulation of ion channels could be through cAMP [8]. G α_q dependent PLC signaling is controversial [9]. Abbreviations: D₁R, dopamine D₁ receptor; AC5, adenylyl cyclase type 5; PKA, protein kinase A; ERK, extracellular-signal-regulated kinase; GRK, G protein-coupled receptor kinase; DARPP-32, Dopamine and cAMP-related phosphoprotein 32KDa; Rap, a small GTPase; CREB, cAMP response element-binding protein; PLC, phospholipase C.

G proteins are a family of proteins made up of subunits G α , G β , and G γ . D₁Rs stimulate adenylyl cyclase primarily through G α_s . The G α_s family is comprised of G α_s and G α_{olf} , the latter named for its predominant expression in the olfactory system. Studies on G α_{olf} knock-out mice suggested that G α_{olf} may play an essential role in D₁R-mediated cAMP accumulation [10,11]. G α_{olf} knock-out mice showed no hyperlocomotor response to the D₁ agonist SKF81297, and their striatal D₁Rs had decreased affinity for dopamine. The adenylyl cyclase response to dopamine in the caudate/putamen and nucleus accumbens were also decreased dramatically, but prefrontal cortex signaling remained unaffected. Studies on the striatum of Parkinson's disease patients also suggested that G α_{olf} plays the essential role in D₁R-mediated cAMP [12]. The hallmark of Parkinson's disease is decreased dopamine in the striatum. Interestingly, G α_{olf} in this region is less abundant in Parkinson's patients, whereas G α_s is highly expressed. The combination of dopamine deficiency and G α_{olf} being sparsely expressed suggested that the coupling of D₁Rs to adenylyl cyclase is mediated primarily by G α_{olf} or the switch/shift between G α_s and G α_{olf} .

The mRNA of other G proteins are also expressed in the striatum. For example, G protein γ_7 subunit mRNA was detected within rat brain areas that aligned with striatum-enriched adenylyl cyclase, dopamine receptors, and G α_{olf} . This suggested that γ_7 may

be part of the $G_{\alpha_{olf}}$ -containing complex that couples dopamine receptors selectively to adenylyl cyclase [13]. Another study used a ribozyme strategy to suppress the expression of the G protein γ_7 subunit in HEK 293 cells stably expressing the human D_1 Rs, and revealed a significant attenuation of D_1 agonist SKF81297-stimulated adenylyl cyclase activity [14]. This evidence supported the potentially important function of the G protein γ_7 subunit on D_1 R-mediated cAMP signaling.

Along with improved understanding of the interaction between D_1 Rs and each G protein subunit, there is ongoing progress regarding functional selectivity of G protein subtype-specific signaling. Although there are currently no D_1 ligands being developed to target specific G protein subunits, such compounds have been reported for the C-C chemokine receptor 5 (CCR5) [3]. Lorenzen et al. studied four chemokine analogs and found that some analogs were super agonists for $G_{q/11}$ activation, whereas other analogs displayed a signaling bias for $G_{i/o}$. Their results demonstrated that ligands can elicit G protein subtype-specific signaling bias and cause receptors to couple preferentially to one subtype of G protein signaling over others. This is inspiring for the pharmacology field. A timely study is needed to investigate this interesting phenomenon since it is reasonable to assume that some dopamine ligands may also engage each G protein subtype differently, providing a more targeted action.

3. β -arrestin-related Signaling and Mitogen-Activated Protein (MAP) Kinase Activation

In addition to G protein-dependent adenylyl cyclase signaling, research has demonstrated the importance of G protein independent β -arrestin-related signaling. Originally, arrestin was thought to only promote receptor desensitization and internalization. It blocks GPCR coupling to G proteins, preventing GPCR's activation (desensitization), or links GPCR to elements of internalization machinery. Starting in the 1970s, Nobel Laureate Robert Lefkowitz and his colleagues made several discoveries related to β -adrenergic receptors, and β -arrestin related signaling is one of their concomitant discoveries. To date, it has been reported widely in several receptor systems, including β -adrenergic [5], opioid [15], cannabinoid [16], angiotensin II type 1 [17], 5-HT_{2A} [18], apelin [19], growth hormone secretagogue [20], sphingosine 1-phosphate [21], and dopamine D_2 -like receptors (D_2 Rs) [22,23]. Regarding D_1 Rs, there are a few reports that suggested the existence of an interaction between D_1 R and β -arrestin and indicated the involvement of MAP kinase phosphorylation (Figure 1), but many of them did not specify or propose D_1 R-mediated β -arrestin signaling.

Chen et al. used co-immunoprecipitation to show that extracellular signal-regulated kinases 1 and 2 (ERK_{1/2}) formed stable heterotrimeric complexes with the D_1 R and β -arrestin2. In cells transfected with the dominant negative mutant of β -arrestin2, however, the formation of such complexes was inhibited substantially [24]. Similarly, Urs et al. utilized a D_1 R knockout mouse model and demonstrated that formation of the β -arrestin2 and ERK_{1/2} complexes was also blunted [25,26]. In contrast, others stimulated striatal or prefrontal cortex D_1 Rs in vivo or in vitro with the D_1 selective agonist SKF38393 and showed ERK_{1/2} was phosphorylated [24,27,28]. These studies provided the initial evidence of D_1 R-mediated β -arrestin signaling and its function through ERK_{1/2}. In addition to ERK_{1/2}, several studies indicated the involvement of other MAP kinases. Zhen et al. showed SKF38393 increased activation of p38 MAP kinase and c-Jun amino-terminal kinase in SK-N-MC neuroblastoma cells that endogenously express D_1 Rs, whereas ERK activity was not affected [29]. This study suggested that D_1 R-related MAP kinase phosphorylation could be cell-type specific.

Another interesting finding is that D_1 R-related MAP kinase phosphorylation may also be dependent of PKA. Studies in parkinsonian mouse models indicated that a PKA substrate, dopamine and cAMP-regulated 32 kDa phosphoprotein (DARPP-32), was critically involved in D_1 R-mediated ERK_{1/2} phosphorylation. A mutation on the phosphorylation site of DARPP-32 reduced activation of ERK, whereas sensitization of DARPP-32 led to increased activation of ERK_{1/2} [6,30]. The selective PKA inhibitor Rp-cAMP eliminated

the activations of ERK_{1/2}, p38 MAP kinase, and c-Jun amino-terminal kinase [27,29]. In contrast, cAMP directly activated a guanine-nucleotide-exchange factor that stimulates Rap GTPase and promotes the MAP kinase cascade [7,31]. These studies suggested there may be potential cross talk between D₁R-mediated cAMP and β -arrestin-related signaling.

4. Regulation of Ca²⁺, K⁺, and Na⁺ Channels

Many studies have indicated D₁R involvement in the regulation of ion channels. Voltage-dependent Ca²⁺ channels (L-, N-, and P/Q-type) play critical roles in balancing intracellular Ca²⁺ concentrations that are key for neurotransmitter release and synaptic plasticity [32]. Using whole-cell voltage-clamp techniques, Surmeier et al. showed that the application of D₁ agonists reduced N- and P-type Ca²⁺ currents, but enhanced L-type currents. The differential regulation of Ca²⁺ currents by D₁ agonists helps to explain the diversity of effects that D₁Rs have on synaptic integration and plasticity [33]. Hernandez-Lopez et al. furthered the study of D₁R effects on L-type Ca²⁺ current. They examined the impact of D₁ agonists at depolarized and hyperpolarized membrane potentials and showed that D₁R activation either can inhibit or enhance evoked activity, depending on the level of membrane depolarization. Interestingly, the effects on evoked activity at membrane potentials were blocked by the L-type Ca²⁺ channel antagonists nicardipine or calciseptine, and were occluded by the agonist BayK8644. These data indicated that the D₁R-mediated effects occurred through the L-type Ca²⁺ channel [34]. For N-type Ca²⁺ channels, coimmunoprecipitation showed the existence of a D₁R-N-type Ca²⁺ channel signaling complex in the prefrontal cortex. This complex had a direct receptor-channel interaction. D₁ agonists not only regulated N-type Ca²⁺ channel distribution but also inhibited influx Ca²⁺ current. Consequently, neuronal transduction was attenuated [35,36]. The cAMP/PKA/DARPP-32 signaling cascade appeared to mediate these effects on Ca²⁺ channels, as cyclic AMP analogs mimicked the effects of D₁ agonists [33].

The D₁ agonist SKF81297 or SKF82957 in combination with the D₂ agonist quinpirole increased spike firing of nucleus accumbens neurons via inhibition of a slow A-type K⁺ current. This enhancement was prevented by inhibitors of PKA or G _{$\beta\gamma$} and enabled by intracellular perfusion with G _{$\beta\gamma$} . These data suggested that the underlying mechanism of D₁R and D₂R cooperativity in mediating the slow A-type K⁺ current was by activation of specific subtypes of adenylyl cyclases released by G _{$\beta\gamma$} from the G_{i/o}-linked D₂R in combination with G _{α_s} -linked D₁R [37]. The D₁ agonists SKF81297 or dihydrexidine induced prolonged membrane depolarization and excitability of fast-spiking interneurons in the prefrontal cortex. Voltage-clamp analyses revealed that this mimicked dopamine-suppressed inward rectifying K⁺ current and can be reduced by the D₁ antagonist SCH23390 [38–41]. Although the precise mechanism underlying D₁R-mediated K⁺ current changes has not been fully understood, studies have suggested possible options: the direct interaction of cAMP with K⁺ channels and the involvement of D₁R-mediated cAMP/PKA signaling. The first reason is that the effect of D₁R stimulation on K⁺ current can be mimicked by the adenylyl cyclase activator forskolin and the active cAMP analog Sp-cAMP. The second reason is that the inhibition of PKA with either PKI, Rp-cAMP, or the protein phosphatase inhibitor okadaic acid abolished D₁R modulation [40,41].

D₁Rs also appear to impact Na⁺ channels. For example, the D₁ agonist SKF38393 reduced the peak Na⁺ current amplitude in rat striatal neurons and subsequently depressed striatal neuron excitability. These effects were reversed by the D₁ antagonist SCH23390 [42,43]. Intracellular loading of PKA mimicked D₁R-mediated Na⁺ current inhibition, and diffusion of the PKA inhibitor PKI into the cytosol of neurons blocked it, suggesting the involvement of PKA [44]. Schiffmann et al. suggested the critical role of phosphorylated DARPP-32 as part of this pathway since its injection reduced the Na⁺ current amplitude [45]. This line of evidence suggested that D₁R regulation of ion channels may be a subsequent event of D₁-mediated G protein-dependent cAMP signaling. On the other hand, Cantrell et al. reported that phosphorylation of Ser573 on the Na⁺ channel α subunit was critical for D₁R-mediated effects on the Na⁺ current since this site was phos-

phorylated by D₁R activation [46]. Since the structure of the ion channel itself potentially could play a critical role, as shown by the Ser 573 study, it is also reasonable to assume that there is a more “direct” interaction between the D₁R and ion channel. A timely study is needed to investigate this interesting hypothesis. More importantly, if some dopamine ligands can engage each ion channel differently, they may provide a more targeted action and potentially lead to better therapeutic implications.

5. Phospholipase C (PLC) Activation

D₁R-mediated PLC signaling was once proposed as a novel target, but controversies occurred, and it now is considered to be purported. The possibility that D₁Rs may function through PLC first was reported in a series of studies on adenylyl cyclase type 5 (AC5), a dopamine sensitive-adenylyl cyclase. Genetic disruption of the AC5 isoform led to loss of adenylyl cyclase activity after administering the D₁ agonist SKF38393, and this was accompanied by a decrease in the expression of G α_s . AC5 null mice also showed parkinsonian-like motor dysfunction. Interestingly, administration of the partial D₁ agonist SKF38393 improved some of the symptoms, suggesting compensation of D₁ signaling outside the G α_s mechanism and beyond adenylyl cyclase [47,48]. G α_q -mediated PLC activation and subsequent Ca²⁺ elevation as a non-cyclase signaling for D₁Rs was then proposed to explain D₁R-mediated motor behaviors of the null mice. SKF38393 increased G α_q protein binding to the D₁R in the striatum, suggesting the possible role of the G α_q protein in D₁R-mediated PLC activation [49]. Several studies indicated that SKF38393 activated PLC in brain slices, and this action was inhibited selectively by the D₁ antagonist SCH23390. In addition, dopamine-induced inward Ca²⁺ current was mimicked by the administration of SKF38393 and blocked by SCH23390 [50–52].

The most supportive evidence for D₁R-mediated PLC signaling came from studies using the D₁ ligand SKF83959 that has small effects on adenylyl cyclase but strong efficacy for PLC activation. Interestingly, it induced contralateral rotations in the unilateral 6-OHDA-lesioned parkinsonian rat model, and the rotations were completely blocked by the D₁ antagonist SCH23390 [53,54]. The involvement of the D₁R in PLC signaling, however, is still controversial [9] because the behavioral effects of SKF83959 can be explained by several other mechanisms. First, SKF83959 is still a typical partial agonist for adenylyl cyclase [55]. Second, non-specific effects on other receptors also could explain the behavioral effects of SKF83959 since it can bind to several GPCRs in micromolar concentrations [56]. Third, it is postulated that D₁Rs and D₂Rs form a D₁/D₂ heterodimer. Heterodimers have been shown to play a role in functional selectivity in several other GPCR systems [57,58], including the D₂/neurotensin NTS1 receptor complex [59] and the D₂/trace amine-associated receptor 1 heterodimer [60]. It is very tantalizing to think that the D₁/D₂ heterodimer led to PLC signaling. D₁Rs and D₂Rs, however, are seldom co-expressed in striatal neurons [61–65], suggesting that the heterodimer mechanism is likely not a major contributor. Collectively, the evidence suggests that D₁Rs actually may be independent of PLC activation.

6. Insight on Functional Selectivity through the Implications of D₁ Signaling

Dopamine receptors are highly expressed in the brain. The densest area is forebrain where the major dopaminergic terminal fields occur including caudate-putamen and nucleus accumbens. The midbrain (i.e., substantia nigra and ventral tegmental area) also has a high density of dopamine receptors. Olfactory, limbic, and brainstem areas have moderate densities of dopamine receptors. The cerebral cortex has a relatively light density of dopamine receptors but those that are there have important functional implications. In general, the density of D₁Rs (including D₁ and D₅) is higher than that of D₂Rs (D₂, D₃ and D₄), especially in the cortex where there is a significantly higher overall density and different laminar patterning of D₁R compared to D₂R. The D₁R is preferentially distributed in deeper cortex layers and is proportionally more widespread and expressed within local GABAergic interneuron populations. In the basal ganglia where the density of dopamine

receptors is the highest, the segregation of D₁R and D₂R is more distinct, with <10% overlap. The GABAergic medium spiny projection neurons of the striatum express D₁R in the direct pathway and D₂R in the indirect pathway. These two parallel and segregated pathways form the outflow of the basal ganglia to regulate thalamocortical circuitry [66]. Dysfunction of dopamine receptors in these brain areas play causal roles in many neurological disorders. Therefore, targeting D₁Rs for therapeutic intervention is attractive. In this section, we try to differentiate D₁ signaling by highlighting some reports that in retrospect have contributed to the understanding of functional selectivity.

There has been a long history between D₁R-mediated cAMP and Parkinson's disease. AC5 is highly concentrated in the striatum. Genetic ablation of the AC5 gene eliminated adenylate cyclase activity stimulated by D₁ agonists in the striatum, and induced parkinsonian-like motor dysfunction. These findings supported the involvement of D₁R-mediated AC5 activation in the motor symptoms of Parkinson's disease [47,48,67]. AC5-produced striatal cAMP binds to the regulatory subunits of PKA that then phosphorylates various proteins such as DARPP-32 and cAMP response element-binding protein (CREB). Although how this signaling leads to D₁-mediated behavioral effects is still unclear, these downstream molecules are involved in the regulation of gene expression [4]. These lines of evidence encouraged the development of functionally selective dopamine ligands whose cAMP signaling can be biased to the PKA subunit to provide a more targeted action improved therapeutic index.

Recent studies on D₁R-mediated β -arrestin have yielded several impressive clinical implications. Urs et al. reported that the D₁R-dependent, β -arrestin-related ERK signal cascade affected morphine-induced psychomotor activation but not reward [24,25], suggesting a separation of therapeutics (e.g., analgesic) from side effects (e.g., addiction). By analyzing transcriptional signatures in humans and mice, Labonte et al. reported that D₁R-mediated β -arrestin signaling through ERK may effect sex-specific depression [68]. Several studies on rats or mice with mutations that eliminate β -arrestin recruitment showed less locomotor activity [69], more dyskinesia-like behavior [26], enhanced adiposity [20], and impaired memory reconsolidation [70]. Recently, our team used a pair of D₁ agonists with distinct signaling profiles at β -arrestin recruitment to evaluate rodent behavior in a working memory-related T-maze task [71]. We showed subtle but significant behavioral variation associated with the level of β -arrestin recruitment, suggesting a promising implication of β -arrestin-selective D₁ agonists on cognitive improvement. Interestingly, there were also clinical implications reported from ligands that bias against β -arrestin. Jiang et al. and other groups reported that β -arrestin activation was related to β -amyloid-induced cognitive impairment [72–74]. Since β -amyloid is one of the key players in Alzheimer's disease, this finding implies that bias against β -arrestin could be a target for limiting β -amyloid-induced cognitive impairment. D₁ agonists that have less β -arrestin activity may be novel therapeutics. Functionally selective D₁ agonists that have less β -arrestin activity may have less side effects in Parkinson's disease because ERK_{1/2} activation correlated with levodopa-induced dyskinesia, whereas blocking ERK_{1/2} activation significantly decreased it [30]. Indeed, recent phase III Parkinson's disease and phase IIa schizophrenia trials using novel D₁ agonists with almost no β -arrestin activity have shown highly positive clinical indications [75–80].

It is encouraging to see D₁R signaling has several clinical implications, even though some results seemed to contradict each other. In fact, the concept of functional selectivity was born in part to deal with the fact that different types of bias at a signaling pathway lead to benefits or disadvantages depending on different conditions. Essentially, functional selectivity was founded on the idea of precise targeting. In other words, the bias at one receptor's whole signaling complex could be adjusted based on different applications to magnify merit.

7. Progress on the Structural Biology of Dopamine Receptors

Progress on the structural biology of dopamine receptors has a large impact on the theory of functional selectivity and the discovery of subtype selective ligands, although it is rather slow for D₁Rs. The crystal structure of D₁R complexed with a G protein and a non-catechol agonist was reported only recently [81], although predictions of the 3D structure of human D₂Rs, the binding site, and binding affinities for agonists and antagonists have been around since the 2000s [82]. Over the intervening years, several crystal structures of ligand-bound D₂R, D₃R, D₄R, and D₂R-G_i complexes were reported [83–85]. By studying selected transmembrane-5 serine mutations, Fowler et al. showed that receptor conformations were involved in D_{2L}R functional selectivity [86]. Using docking simulations and site-directed mutagenesis, Zhang et al. not only reported the crystal structure of the human angiotensin II type 1 receptor in complex with one of its inverse agonists but also identified specific interactions between the angiotensin II type 1 receptor and different ligands. This provided support for the structural basis of ligand recognition and functional selectivity [87].

The heterodimer theory was also enhanced by structural biology progress. Crystal structures of receptor–ligand complexes allow for rational design of novel molecular probes and drugs. Following this strategy, bivalent ligands were synthesized and investigated for the D₂R/neurotensin NTS1 receptor heterodimer. The results indicated a strong, predominantly NTS1 receptor-mediated β -arrestin-2 recruitment in D₂R/NTS1 receptor-co-expressing cells [59]. There have been several other studies on the functional selectivity of GPCR heterodimerization [57,58] but because of the sparse co-expression of D₁Rs and D₂Rs in the same neuron [61–65], it still is unclear whether the D₁/D₂ heterodimer plays a critical role in cell signaling or overt behaviors.

8. Potential of Receptor Localization-Related Functional Selectivity

It is commonly known that dopamine receptors function differently in distinctive brain areas. Although there is not yet many focused studies to specifically investigate this, the possibility is high that functional selectivity is involved as a part of the underlying mechanism. More intriguingly, recent studies have shown that besides the primary effects in the central nervous system, dopamine also acts in the pancreas as a peripheral regulator of metabolism, and this action is functionally biased at D₂Rs [88]. This suggests that the receptor localization-related functional selectivity may be a widespread phenomenon. GPCRs not only function at the plasma membrane but also at various cellular organelles including endosomes, mitochondria, and Golgi [89]. Although D₁-related studies are sparse, it will not be a surprise that future studies show D₁ functional selectivity in different organelles. Location bias is an emerging paradigm in GPCR biology and drug discovery. This is particularly true for endosomes regarding D₁Rs. D₁Rs start to internalize through endosomes where β -arrestin is recruited. It has been shown that endocytosis promotes rapid D₁ signaling [90]. This could be in line with the initial signal at the plasma membrane, but there are studies showing GPCR signaling via heterotrimeric G proteins from endosomes [91]. Therefore, it will not be unexpected that D₁ has signaling bias toward β -arrestin at endosomes, but more studies are needed for this topic.

9. Functionally Selective D₁ Ligands: Pharmacological Retrospect

Functional selectivity is currently a hot topic in the drug discovery field. Recently, several novel, functionally selective D₁ ligands were reported [92,93]. They all were biased toward cAMP compared to β -arrestin signaling. Functional selectivity, at the extreme, has a ligand act as both an agonist and antagonist in different cellular functions at a single receptor [1]. An example would be functioning as an agonist at cAMP and an antagonist at β -arrestin (Figure 2). For many dopamine ligands, however, it is more common to activate all signaling pathways but to different degrees. This incomplete/subtle functional selectivity was first reported by our group using a series of full and partial D₁ agonists [71]. Full and partial agonists were defined by activation of the canonical D₁ signaling pathway—the intrinsic activity at stimulating adenylate cyclase and producing cAMP. It is noteworthy

that a retrospective review of some “traditional/classical” D₁ ligands may have revealed that they are functionally selective even if this was not appreciated at the time of publication, as highlighted by our group [71].

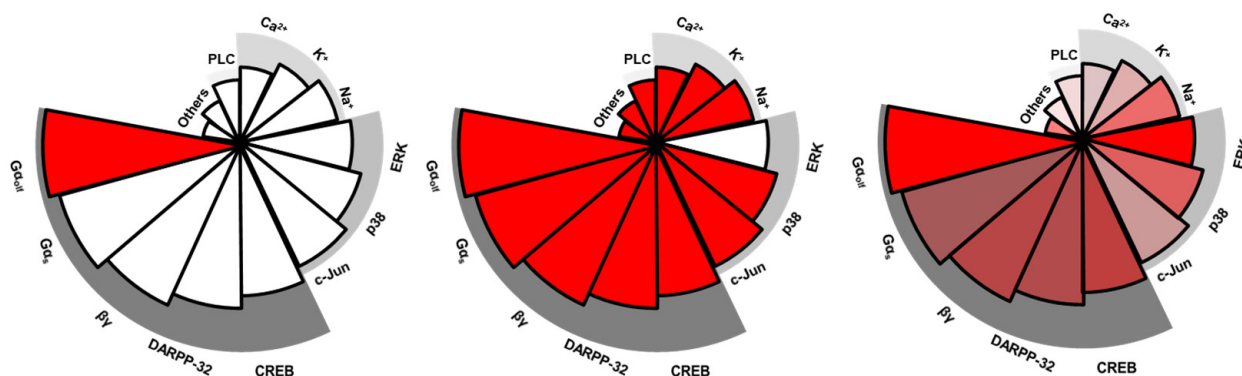


Figure 2. Diagram of functional selectivity. Complete functional selectivity, at the extreme, is a ligand acting as both an agonist and an antagonist at different functions at a single receptor. The left panel exemplifies complete bias with a ligand functioning as an agonist (red color) only at G α_{o1f} -mediated cAMP activation. The middle panel exemplifies another complete bias with a ligand functioning as an antagonist (blank) only at β -arrestin-related ERK activation. Incomplete/subtle functional selectivity more commonly is seen in many dopamine ligands. The right panel exemplifies mild bias since all signaling pathways are activated but to different degrees. Each pie represents a signaling pathway; red color indicates pathways are activated but different transparencies reflect they are activated to different levels (e.g., full vs. partial). Note the signaling pathways, such as G α_{o1f} -mediated cAMP activation or β -arrestin related ERK activation, are only examples.

Lewis et al. compared the intrinsic activities at adenylyate cyclase for a series of structurally dissimilar full and partial D₁ agonists and their ability to cause functional desensitization [94]. Surprisingly at the time, they found a dissociation between these two activities. For example, three full D₁ agonists (dihydroxidine, SKF82958, A77636) caused homologous desensitization of the D₁R in vitro to the same extent as dopamine, whereas two other full agonists (dinapsoline, A68930) and all the partial agonists (SKF38393, pergolide, LSD) caused only partial desensitization that was homologous, but not associated with PKA-induced phosphorylation. Ryman-Rasmussen et al. later tested 13 D₁ agonists and showed that internalization efficacy was independent of either agonist affinity or chemotype [95]. For example, four agonists from two chemotypes were able to activate adenylyate cyclase fully without inducing internalization. A follow-up study further confirmed that ligand-specific differential effects on receptor recycling involved aspects of D₁Rs that are distal from the ligand-binding domain [96]. Since receptor recycling (i.e., desensitization, internalization) is initiated by β -arrestin recruitment, it is logical to conclude that a retrospective review of these data actually provides proof that these compounds have some degree of functional selectivity between canonical cAMP signaling and novel β -arrestin-related signaling. Moreover, these data are consistent with the hypothesis that functional selectivity reflects subtle ligand-induced conformational changes, as opposed to simple agonist trafficking among discrete receptor active states.

10. Prospect on Future Studies of Function Selectivity

Functional selectivity was first reported as a pharmacological phenomenon. Later studies using behavioral and neurophysiological methods, and recent clinical trials, have led to improved understanding of functionally selective drugs and provided significant insight on how to further investigate this “so called” pharmacological phenomenon. The earliest example of functional selectivity was for a series of D₂ ligands that were full agonists at adenylyate cyclase but antagonists at other functions [97–99]. The translated in vivo behavioral effects of these highly functionally selective compounds indicated they were uncharacteristic of typical D₂ agonists [100,101]. Contemporaneous studies of OPC-

14597 (later named aripiprazole) showed that its unusual properties most likely involved functional selectivity at the D₂Rs and possibly 5-HT_{1A} receptors [1,102,103], contrary to later views of it functioning as a simple partial agonist. Spurred by studies emerging at the same time in serotonin [104], angiotensin [17], and opioid [105] systems, both basic research and drug discovery have exploded in the past decade with numerous exciting findings on functional selectivity. One timely area relates to the opioid epidemic in which the search for functionally selective opioid receptor ligands indicated superior analgesic action with decreased addictive or other unwanted properties [106–109]. Recently, our group reported a landmark neurophysiological study on the functional selectivity of D₁R-mediated cAMP and β -arrestin signaling. Using a pair of D₁ agonists with distinct signaling profiles, we evaluated rodent behavior in a T-maze task and examined how this was associated with neural activities in the prefrontal cortex [71]. We showed significant neurophysiological changes correlated with the level of β -arrestin recruitment. These results indicated the feasibility of using neurophysiological measurements as markers for studying D₁R functional selectivity. It is encouraging that the field has discovered more ways to investigate functional selectivity—not only by pharmacological means but also behavioral and neurophysiological methods. These interdisciplinary approaches improve the innovation and development of more functionally selective D₁ ligands as better therapeutics.

11. Summary

The understanding of functional selectivity has evolved over the years in conjunction with advances in improved knowledge of fundamental receptor signaling and complexes. With the discovery of many novel signaling or sub-pathways related to D₁Rs, studies related to grasping the breadth of D₁R functional selectivity are expanding. Even though some reports at the time of publishing did not focus on functional selectivity, a retrospective review of their findings indicate they contributed to this field. More importantly, many studies showed positive implications for each unique D₁ signaling pathway, suggesting that functional selectivity could be a promising strategy for drug discovery. Furthermore, retrospective pharmacological review revealed that many D₁ ligands have some degree of mild functional selectivity. Moreover, novel compounds with extreme bias at D₁ signaling were reported recently. Collectively, these data show that the development of precision medicine with the use of functionally selective D₁ ligands is a promising direction to pursue.

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