Neuron, Vol. 47, 335–338, August 4, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.neuron.2005.07.015

The Dopamine D2 Receptor: New Surprises from an Old Friend

Minireview

Antonello Bonci[1,2,*] and F. Woodward Hopf[1]
[1]Ernest Gallo Clinic and Research Center
Department of Neurology
University of California, San Francisco
Emeryville, California 94608
[2]Wheeler Center for the Neurobiology of Addiction
University of California, San Francisco
San Francisco, California 94110

Drugs acting at dopamine D2 receptors (D2R) are commonly used to alleviate symptoms produced by diseases such as Parkinson's disease, schizophrenia, and depression. A limitation to the use of these drugs is that they sometimes afflict patients with severe side effects. This review discusses recent evidence for several proteins that represent novel mediators of the downstream consequences of D2R activation, since selective targeting of particular D2R-mediated signaling pathways could lead to the development of improved treatments for these devastating diseases.

The goal of this review is to briefly summarize our current knowledge about dopamine D2 receptors (D2Rs), in light of two recent papers that have advanced our understanding of the complexity of the intracellular physiology that is coordinated by this receptor. Dopamine receptors are G protein-coupled receptors and belong to two main families (reviewed in Missale et al., 1998; Neve et al., 2004): the D1-like receptor subfamily (D1R), including the D1 and D5 receptors; and the D2-like subfamily, including the D2, D3, and D4 receptors. D1Rs activate adenylyl cyclase (AC) via their coupling to G_s/G_{olf}, while D2Rs are $G_{i/o}$ linked and release $G_{\alpha i/o}$ and $G_{\beta\gamma}$ subunits. Classically, D2R function has been thought of in terms of antagonism of cAMP-dependent signaling, where $G_{\alpha i}$ subunits bind to and inhibit adenylyl cyclases, preventing production of cAMP and activation of protein kinase A (PKA) (Figure 1A). In this view, the primary action of dopamine is to activate neurons through the D1 receptor, and this activation is reduced by concurrent activation of the D2 receptor. Although some neurons have only D2 or only D1 receptors, D2/D1 antagonism is widely observed. In striatal neurons, DARPP-32 is a primary target of PKA, and DARPP-32 is involved in many dopaminergic actions, including D2R inhibition of the cAMP-dependent system.

D2 receptors also alter intracellular signaling through $G_{\beta\gamma}$ subunits, which can act at a number of intracellular targets (Figure 1B) (reviewed in Neve et al., 2004). In addition to direct interaction of $G_{\beta\gamma}$ with several types of ion channels, $G_{\beta\gamma}$ subunits facilitate calcium release from intracellular calcium stores. To further illustrate the complexity of the downstream effectors activated by D2R, $G_{\beta\gamma}$ subunits can activate the MAP kinase system through several different pathways, which can involve the phosphinositide 3-kinase, Ras, and transactivation of a growth factor receptor.

The activity of the D2R itself is regulated by desensitization, where continuous agonist application results in phosphorylation of the D2R (e.g., by the G protein receptor kinase GRK2), leading to uncoupling of receptors from G protein activation and promotion of binding of arrestin and receptor internalization (Gainetdinov et al., 2004) (Figure 1B). Also, as described below, Beaulieu et al. (2005) provide novel evidence that β-arrestin 2 can also facilitate some aspects of D2R signaling.

From an anatomical standpoint, the D2R is present in many areas of the central nervous system, but it is preferentially located in the substantia nigra pars compacta, the ventral tegmental area, the striatum (which includes the nucleus accumbens shell and core and the dorsal striatum), olfactory tubercule, and the pituitary gland (reviewed in Missale et al., 1998). Functionally, like many G protein-coupled receptors, D2R can be located both presynaptically, regulating release of dopamine and other neurotransmitters, and postsynaptically, where it can exert a variety of functions, ranging from inhibition of long-term depression at midbrain excitatory synapses, to inhibition of calcium channels, to control of pacemaker activity and resting potential through activation of GIRK channels (Hopf et al., 2003; Jones and Bonci, 2005; and references therein).

Role of Dopamine D2 Receptors in Neurological and Psychiatric Diseases

D2R regulatory molecules are also interesting as potential therapeutic targets because dopaminergic dysregulation is implicated in a number of neurological and psychiatric conditions, but no change in dopamine receptor abundance has been observed. Dysregulation of dopamine signaling could also be due to alterations of the D2R itself: while a missense mutation in the DRD2 gene causes myoclonus dystonia, other mutations at D2Rs have been associated with a variety of neurological and psychiatric diseases ranging from Parkinson's disease to substance abuse, schizophrenia, and bipolar disorders (for a complete list of studies, please go to http://geneticassociationdb.nih.gov).

Of particular relevance to this brief review is the potential role of abnormal D2R activity in mediating symptoms associated with schizophrenia and depression. D2R antagonists have been pursued for a long time as antipsychotic agents. Although there is general consensus that every antipsychotic drug must inhibit D2Rs to be clinically effective for schizophrenia, there is a clear dissociation between the relatively low therapeutic benefit and their high pharmacological selectivity as D2R antagonists (reviewed in Miyamoto et al., 2005). Thus, while extensive research on therapeutic agents acting at D2Rs has been performed, the mechanism underlying the therapeutic properties of these drugs remains to be defined. However, animal studies support a role for the D2R in schizophrenia. Schizophrenia is associated with supersensitivity to dopamine, and a

*Correspondence: bonci@itsa.ucsf.edu

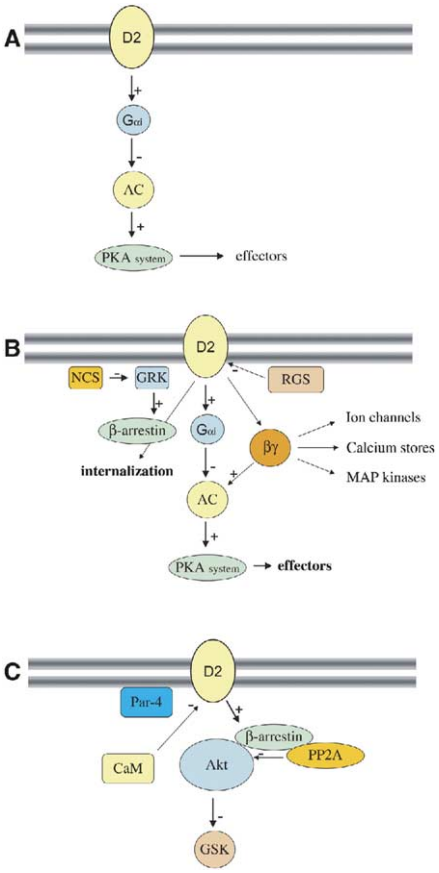

Figure 1. Evolution of the Dopamine D2R-Dependent Intracellular Pathways

Panels (A) and (B) summarize how our knowledge of the dopamine D2R-dependent intracellular pathways has evolved over time, from inhibition of cAMP signaling (A), to signaling through $G_{\alpha i}$ and multiple $G_{\beta\gamma}$-dependent pathways and regulation both by internalization and by molecules such as RGSs (B). Panel (C) refers to the finding from the two papers by Beaulieu et al. (2005) and Park et al. (2005).

number of treatments that enhance dopamine sensitivity in rodents enhance the number of D2Rs in the high-affinity state (Seeman et al., 2005). Further, sensorimotor gating, which is impaired in schizophrenics and disrupted by dopamine in rodents, requires the D2R but not D3R or D4R (Ralph et al., 1999). Thus, a deeper understanding of the complex D2R-dependent intracellular pathways is likely to represent a key step to aid scientists in designing more effective antipsychotics.

A key role for dopamine in some of the symptoms commonly observed in depressed patients, such as anhedonia or decreased motivation, has also been suggested for years (reviewed in Dailly et al., 2004). In animal models of depression, like the forced swim test, the efficacy of the selective dopamine reuptake inhibitor GBR 12909 as an antidepressant has been shown. There is also evidence for a synergistic/cooperative effect of D2R agonists (but also D1R or D3R agonists) with traditional antidepressants that belong to the selective serotonin reuptake inhibitor (SSRI) family, since these SSRIs coadministered with a dopamine agonist significantly improve the rodent's performance on the forced swim test. Human studies provide further support for a role of D2R in depression because some antidepressants act in part by blocking dopamine reuptake and by activating dopamine receptors. In addition, a compensatory upregulation of D2Rs has been observed in the striatum of patients suffering from depression. Taken together, these studies suggest that enhancing dopamine signaling might represent a promising complementary target to traditional antidepressants, especially when anhedonia and apathy are primary symptoms.

Regulation of D2Rs by Intracellular Binding Partners

It has become clear in recent years that few receptors act in isolation, but instead exist in a complex with regulatory and scaffolding molecules. New promising insights come from two recent studies by Beaulieu et al. (2005) and Park et al. (2005), both of which have identified new intracellular proteins and pathways that are modulated by D2R activity.

The study by Beaulieu and colleagues provides evidence that a member of the D2R receptor subfamily inhibits the activity of the serine threonine kinase Akt through a β-arrestin 2-dependent mechanism and that this effect occurs through a newly discovered β-arrestin/kinase/phosphatase signaling complex (Figure 1C) that is independent from the traditional cAMP-dependent pathway. This suggests that β-arrestin plays a role in signal transduction, in addition to its canonical role in receptor internalization, through a complex composed of the intracellular proteins Akt, β-arrestin 2, and phosphatase PP2A. Combined with results from a previous study by the same group (Beaulieu et al., 2004), these results demonstrate that β-arrestin 2 is critical in regulation of Akt and its downstream target glycogen synthase kinase (GSK) (Figure 1C). The authors also show that activation of D2-like receptors is unable to modulate the activity of the protein Akt in the striatum in these mutant mice, suggesting that β-arrestin 2 appears to be critical for the dopamine-dependent dephosphorylation of Akt by allowing a specific association between Akt and the phosphatase PP2A (Figure 1C). Behaviorally, genetic knockout of either β-arrestin 2 or GSK reduces the acute locomotor response to psychostimulants, and β-arrestin 2 knockouts lack the increased exploratory activity in novel environments that is present in normal mice. This study is particularly exciting in that it opens new avenues for investigation of D2R-mediated, cAMP-independent intracellular pathways. In addition, alterations in Akt and GSK may be implicated in schizophrenia (Emamian et al., 2004), and Beaulieu and colleagues suggest that the D2R/Akt/β-arrestin pathway they have identified might contribute to the dopaminergic dysregulation thought to occur in schizophrenic patients.

The study by Park and colleagues shows that prostate apoptosis response 4 (Par-4) is a novel binding partner of the D2R. Par-4 has been implicated in apoptosis and neuronal death, but its presence in synapses suggests other unidentified roles in neuronal physiology. Par-4 interacts with the D2R at the calmodulin binding motif in the third cytoplasmic loop of the D2R (Figure 1C). Calmodulin (CM) binding to the D2R displaces Par-4 and inhibits D2R activation of G proteins.

Thus, Par-4 might serve as a counterbalance to this calcium/CM-mediated inhibition of D2R function. Loss of Par-4, either through RNAi in culture or in a mutant mouse with a deletion in the leucine zipper domain that binds the D2R (Par-4ΔLZ), blocks D2R action and thereby facilitates D1R-mediated activation of adenylyl cyclase and CREB phosphorylation. Most interesting is the observation that Par-4ΔLZ mice exhibit a depression-like syndrome. For example, in the forced swim test, rodents swim around for a while, but eventually give up and become immobile. A shorter latency to immobility ("giving up sooner") is considered consistent with a depression-like syndrome, as it is reversed by most antidepressants. Par-4ΔLZ mice exhibit significantly shorter latencies to immobility relative to wild-type mice. Importantly, Par-4ΔLZ mice do not exhibit differences in tests of anxiety, suggesting that earlier immobility is not secondary to responsiveness to anxiety and stress.

To understand the present results in the context of depression, it is critical to understand the altered cellular mechanism within neurons that mediates depression-like behaviors. It is clear that a number of neurochemical systems can be altered during depression and might contribute to the clinical syndromes observed in humans (Manji et al., 2001; Nestler et al., 2002). As mentioned above, links between dopamine signaling and depression are tantalizing but still somewhat tenuous. Certainly dopamine is important for motivation and responsiveness to salient stimuli, and decreased motivation and loss of responsiveness can be observed in depressed humans. Depression in Parkinson's patients also supports the idea that reduced dopamine function is causally implicated in the manifestation of depression. Thus, impaired D2R function secondary to loss of Par-4 represents an attractive hypothesis for the presence of a depression-like syndrome in Par-4ΔLZ mice. However, in striatal neurons, loss of Par-4 is associated with decreased D2R function, but also with facilitation of D1 receptor activation of the PKA system and enhanced CREB phosphorylation relative to wild-type neurons. Thus, depression-like symptoms in Par-4ΔLZ mice might reflect an altered balance between D1R and D2R signaling rather than simply decreased dopaminergic activation.

Relating D2 or D1 receptor function to depression is complicated by the fact that the exact role of D2 and D1 in reward and motivation is still a matter of debate, since activation of these receptors produces a wide range of functional responses that are dependent upon the activity state of the neurons. For example, a number of studies have found a role for both D2 and D1 in the nucleus accumbens in motivation and goal-directed behaviors (Yun et al., 2004; and see references in Hopf et al., 2003). Brain slice electrophysiology experiments have found D1/D2 synergy in several brain regions, including the nucleus accumbens (Hopf et al., 2003). Interestingly, Par-4 binds an atypical isoform of PKC (aPKC) in addition to the D2R, and aPKCs are necessary for the D2 (and D1) receptor-dependent excitation of nucleus accumbens shell neurons (Hopf et al., 2005). Dopamine receptor activity in regions other than the nucleus accumbens can also contribute to motivated behaviors, but we would like to emphasize here that there are still significant gaps in our understanding of the relationship between D2 and D1 receptors and basic motivational drives, let alone depression.

The results from these papers are particularly interesting because it is possible that selective restoration of β-arrestin 2 or Par-4 function within particular brain regions of β-arrestin 2 or Par-4 mutant mice, perhaps using a viral approach, would help in identification of the brain regions responsible for schizophrenia-like or depression-like symptoms. The ability to pinpoint the brain region(s) responsible for these behavioral abnormalities would significantly advance our understanding of the critical neuronal pathways involved and perhaps suggest these new pathways as novel therapeutic interventions. Along this line, the observation that Par-4 did not bind to other serotoninergic and adrenergic G_i-linked receptors and that cAMP accumulation in response to these neuromodulators was not altered in Par-4ΔLZ mice is encouraging and strengthens the possible specificity of the interaction of Par-4 and the D2R and the relationship between the D2R and depression.

Interesting questions remain about the exact molecular mechanism through which Par-4 regulates D2R function. For example, Kabbani et al. (2002) demonstrated that the protein neuronal calcium sensor 1 (NCS-1), which had been previously shown to regulate several aspects of neurotransmission, can be coimmunoprecipitated from striatal membranes with the D2R and with GRK2. Further, Kabbani et al. provided evidence that NCS-1 can reduce D2R phosphorylation and internalization and can facilitate D2R-mediated inhibition of adenylyl cyclase activation. NCS-1 regulation of D2R is likely due to a calcium-dependent association of NCS-1 with GRK2, suggesting that NCS-1 regulates D2R function through inhibition of GRK2, analogous to NCS-1 regulation of rhodopsin by inhibition of GRK1.

It will be interesting to determine whether Par-4 might regulate D2R through altered phosphorylation or internalization through interaction with NCS-1, GRK, or by some other mechanism. For example, Par-4 could regulate D2Rs through RGS proteins, several of which have been shown to attenuate D2R function (Rahman et al., 2003; Jeanneteau et al., 2004). In addition, Par-4 facilitates actin bundling and might perform a scaffolding function, perhaps similar to the requirement for the actin binding protein filamin for plasma membrane localization of D2R (Lin et al., 2001). The GSK/Akt pathway can also regulate the actin cytoskeleton by enhancing actin filament stability (Owen and Gordon-Weeks, 2003), suggesting that Par-4 may regulate D2R function through modulation of the β-arrestin 2/Akt/PP2A pathway described by Beaulieu et al. (2005). This would be particularly interesting because the D2R-mediated effects in Beaulieu's study are not apparent until after 30 or more minutes, while Par-4 regulation of D2R function is apparent earlier, suggesting that D2R activation of GSK might be regulated on different time scales by different intracellular signaling mechanisms.

Park et al. also suggest, based on the observation that motor coordination is not disrupted in the Par-4ΔLZ mice but is disrupted in the D2 knockout or after OHDA lesions, that Par-4 may mediate only some of the functions of the D2R. This raises the interesting ques-

tion of whether there are different populations of D2Rs, linked to different effectors via selective scaffolding/regulatory proteins. These different populations might represent variation in isoforms, affinity states, multimerization, or glycosylation-state (see Lee et al., 2003; Neve et al., 2004; Seeman et al., 2005; references in Park et al., 2005) or might involve the same D2R variants, which are segregated functionally into different intracellular microdomains. However, according to Beaulieu et al., removal of β-arrestin 2 alters regulation of both cAMP-independent and cAMP-dependent signaling. Thus, different molecules might contribute to one or multiple effector pathways of the D2R.

It is also important to note that the majority of studies directly examining intramolecular interactions have been performed in immortalized cells or neuronal cultures and often involve overexpression of the molecules of interest. The more difficult studies showing similar interactions in intact neurons would strengthen the in vivo relevance of such observations. Behavioral experiments involving knockout mice or viral-mediated genetic manipulation are very helpful in this regard, although it is more difficult in the intact brain to be certain that removal of a particular molecule (e.g., Par-4) is having its behavioral effect through a particular target (e.g., the D2R). In many cases, brain slice experiments could also be very useful in discerning the details of molecular interactions. For example, Bartlett et al. (2005) recently showed that the protein GASP plays a critical role in activation-dependent reduction in D2R function by targeting internalized D2R to the degradative pathway. Further, patch clamp electrophysiology was used to demonstrate that prolonged D2R activation led to persistent loss of D2R function in VTA DA neurons, but that intracellular perfusion with an antibody that blocks the function of GASP allowed recovery of D2R function. Because patch clamp allows more precise control of the intracellular milieu (and, for example, the level of free calcium) as well as perfusion of peptides and other proteins, this technique cannot only verify the importance of proposed signaling interactions under physiologically relevant conditions, but also can directly assay the physiological consequences of such signaling in intact neurons.

Conclusions

These studies represent exciting additions to the information relating the function of particular molecules with behavior. In particular, because of the strong link between dopamine dysregulation and several psychiatric disorders, identification of novel pathways for regulation of dopamine receptor function may open intriguing new avenues for understanding the etiology of such disorders and perhaps for the development of novel therapeutic interventions. However, we must temper our enthusiasm in relation to the role of a specific molecule with the knowledge that neuronal signaling in vivo is complex, with many neurochemicals and proteins in multiple brain regions contributing to even the simplest of behaviors. For example, Peter Kalivas et al. (2005) have shown that chronic cocaine self-administration alters the function of several different molecules and that normalization of the function of any one of these molecules is sufficient to reduce reinstatement of cocaine seeking. The lesson here is that multiple molecules likely contribute to both normal and abnormal behavioral states. Thus, it is very exciting when a particular molecule can be so elegantly linked to the expression of interesting behaviors, but it is essential to remember that no molecule can get by without a little help from its friends.

Selected Reading

Beaulieu, J.M., Sotnikova, T.D., Yao, W.D., Kockeritz, L., Woodgett, J.R., Gainetdinov, R.R., and Caron, M.G. (2004). Proc. Natl. Acad. Sci. USA *101*, 5099–5104.

Bartlett, S.E., Enquist, J., Hopf, F.W., Lee, J.H., Gladher, F., Kharazia, V., Mailliard, W.S., Waldhoer, M., Armstrong, R., Bonci, A., and Whistler, J.L. (2005). Proc. Natl. Acad. Sci. USA, in press.

Beaulieu, J.M., Sotnikova, T.J., Marion, S., Lefkowitz, R.J., Gainetdinov, R.R., and Caron, M.G. (2005). Cell *122*, 261–273.

Dailly, E., Chenu, F., Renard, C.E., and Bourin, M. (2004). Fundam. Clin. Pharmacol. *18*, 601–607.

Emamian, E.S., Hall, D., Birnbaum, M.J., Karayiorgou, M., and Gogos, J.A. (2004). Nat. Genet. *36*, 131–137.

Gainetdinov, R.R., Premont, R.T., Bohn, L.M., Lefkowitz, R.J., and Caron, M.G. (2004). Annu. Rev. Neurosci. *27*, 107–144.

Hopf, F.W., Cascini, M.G., Gordon, A.S., Diamond, I., and Bonci, A. (2003). J. Neurosci. *23*, 5079–5087.

Hopf, F.W., Mailliard, W.S., Gonzalez, G.F., Diamond, I., and Bonci, A. (2005). J. Neurosci. *25*, 985–989.

Jeanneteau, F., Guillin, O., Diaz, J., Griffon, N., and Sokoloff, P. (2004). Mol. Biol. Cell *15*, 4926–4937.

Jones, S., and Bonci, A. (2005). Curr. Opin. Pharmacol. *5*, 20–25.

Kabbani, N., Negyessy, L., Lin, R., Goldman-Rakic, P., and Levenson, R. (2002). J. Neurosci. *22*, 8476–8486.

Kalivas, P.W., Volkow, N., and Seamans, J. (2005). Neuron *45*, 647–650.

Lee, S.P., O'Dowd, B.F., and George, S.R. (2003). Life Sci. *74*, 173–180.

Lin, R., Karpa, K., Kabbani, N., Goldman-Rakic, P., and Levenson, R. (2001). Proc. Natl. Acad. Sci. USA *98*, 5258–5263.

Manji, H.K., Drevets, W.C., and Charney, D.S. (2001). Nat. Med. *7*, 541–547.

Missale, C., Nash, S.R., Robinson, S.W., Jaber, M., and Caron, M.G. (1998). Physiol. Rev. *78*, 189–225.

Miyamoto, S., Duncan, G.E., Marx, C.E., and Lieberman, J.A. (2005). Mol. Psychiatry *10*, 79–104.

Nestler, E.J., Barrot, M., DiLeone, R.J., Eisch, A.J., Gold, S.J., and Monteggia, L.M. (2002). Neuron *34*, 13–25.

Neve, K.A., Seamans, J.K., and Trantham-Davidson, H. (2004). J. Recept. Signal Transduct. Res. *24*, 165–205.

Owen, R., and Gordon-Weeks, P.R. (2003). Mol. Cell. Neurosci. *23*, 626–637.

Park, S.K., Nguyen, M.K., Fischer, A., Luke, M.P.-S., Affar, E.B., Dieffenbach, P.B., Tseng, H.-C., Shi, Y., and Tsai, L.-H. (2005). Cell *122*, 275–287.

Rahman, Z., Schwarz, J., Gold, S.J., Zachariou, V., Wein, M.N., Choi, K.H., Kovoor, A., Chen, C.K., DiLeone, R.J., Schwarz, S.C., et al. (2003). Neuron *38*, 941–952.

Ralph, R.J., Varty, G.B., Kelly, M.A., Wang, Y.M., Caron, M.G., Rubinstein, M., Grandy, D.K., Low, M.J., and Geyer, M.A. (1999). J. Neurosci. *19*, 4627–4633.

Seeman, P., Weinshenker, D., Quirion, R., Srivastava, L.K., Bhardwaj, S.K., Grandy, D.K., Premont, R.T., Sotnikova, T.D., Boksa, P., El-Ghundi, M., et al. (2005). Proc. Natl. Acad. Sci. *102*, 3513–3518.

Yun, I.A., Wakabayashi, K.T., Fields, H.L., and Nicola, S.M. (2004). J. Neurosci. *24*, 2923–2933.