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Getting specialized: presynaptic and postsynaptic dopamine D2 receptors

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Summary

Dopamine (DA) signaling controls many physiological functions ranging from locomotion to hormone secretion, and plays a critical role in addiction. DA elevation, for instance in the response to drugs of abuse, simultaneously activates neurons expressing different DA receptors; how responses from diverse neurons/receptors are orchestrated in the generation of behavioral and cellular outcomes, is still not completely defined. Signaling from DA D2 receptors (D2Rs) is a good example to illustrate this complexity. D2Rs have presynaptic and postsynaptic localization and functions, which are shared by two isoforms *in vivo*. Recent results from knockout mice are clarifying the role of site and D2 isoform-specific effects thereby increasing our understanding of how DA modulates neuronal physiology.

Introduction

Responses to natural rewards (i.e. food) and addictive drugs share hedonic properties and elevate dopamine (DA) levels in the mesolimbic system, in areas such as the NAcc, which has been shown to be a preferential anatomical substrate for reward [1–3]. Drugs of abuse exploit the dopaminergic system to elicit their behavioral and cellular effects and by enhancing DA responses facilitate the study of the system.

DA effects are elicited through the interaction with membrane receptors that belong to the G-protein coupled receptor family [4]. Thus, upon drug intake DA signaling, controlled by any of the five DA receptors, is strongly activated leading to stimulation or inhibition of pathways regulated by the D1-like (D1 and D5) and D2-like receptor family (D2, D3 and D4), which translates into activation/inhibition of specific neurons and circuitries. In this article we will focus on the pre- and postsynaptic DA D2 receptor (D2R) mediated signaling and functions *in vivo*.

D2Rs, widely expressed in the brain, are localized both on presynaptic dopaminergic neurons, but also on neurons targeted by dopaminergic afferences (Fig.1). In addition of having a dual localization, D2 receptors are a heterogeneous population formed by two molecularly distinct isoforms, named D2S (S=short) and D2L (L=long) generated by alternative splicing of the same gene [4]. Genetically engineered mice deleted or altered [5–9] in D2Rs expression have been critical in identifying D2R-mediated functions *in vivo* [10]. We will discuss the relative contribution of pre- versus post-synaptic D2R-mediated mechanisms in response to DA elevation generated by drugs of abuse or by DA agonists by comparing results from wild-type (WT) and knock-out mice.

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Signal transduction by D2L and D2S differently affects pre- versus postsynaptic responses

The best-characterized intracellular effect of DA is activation of the cAMP pathway [4]. This pathway is activated through D1-like receptors and inhibited by D2-like receptors. In striatal medium spiny neurons (MSNs), elevation of cAMP level leads to the activation of the protein Kinase A (PKA) [11] and consequently to phosphorylation of a large series of cellular targets and importantly of the DA- and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32), [12] (Fig.1). Blockade of D2R stimulates the PKA-dependent phosphorylation of DARPP-32. This effect is most likely mediated via suppression of the inhibition exerted by D2R on the adenylyl cyclase. Phosphorylation catalysed by PKA on Thr34 converts DARPP-32 into a potent inhibitor of PP-1, thereby amplifying the responses produced by activation of the cAMP/PKA pathway. Importantly, blockade of D2R-mediated signaling produces a motor depressant effect, which is attenuated in DARPP-32 null-mice [13]. Activation of D1Rs increases Thr34 phosphorylation via G_{olf} -mediated stimulation [14]. Conversely, activation of D2Rs decreases DARPP-32 phosphorylation at Thr34 via G_i -mediated inhibition of cAMP production [11]. In addition, D2Rs agonists stimulate protein phosphatase-2B activity, thereby increasing dephosphorylation of DARPP-32 at Thr34 [11].

Interestingly, SKF81297, a D1R agonist, produces a ten-fold increase in the state of phosphorylation of DARPP-32 at Thr34, in WT mice, D2R $-/-$ and D2L $-/-$ mice [15]. Quinpirole, a D2-specific agonist, counteracts the increase in phosphorylation of DARPP-32 at Thr34 produced by the dopamine D1 agonist, in WT, but not in D2R $-/-$ or D2L $-/-$ tissues [15]. This suggests that the D2L isoform is responsible for D2-like receptor-mediated regulation of DARPP-32 phosphorylation in MSNs, thereby demonstrating the specific involvement of this receptor isoform in postsynaptic D2R-mediated signaling.

Conversely in dopaminergic neurons of the substantia nigra (SN) and ventral tegmental area (VTA), the reduction of phosphorylation of tyrosine hydroxylase (TH) on Ser40, induced by dopamine D2 specific agonists, is lost in D2R $-/-$ mice, but preserved in D2L $-/-$ as in WT tissues [15]. Indicating a major D2S-specific presynaptic effect.

The specificity in isoform-mediated presynaptic and postsynaptic functions most likely arises from D2L and D2S ability to interact with different G-proteins and signaling pathways [16, 17] or through isoform-specific and yet to unravel protein-protein interactions.

More recently, the implication of the serine/threonine kinase AKT in the signaling mediated by DA through D2-like receptors has been reported [18]. Activation of this pathway is cAMP-independent and mediated through the formation of a macromolecular complex containing at least three proteins, the scaffolding protein β -arrestin 2, AKT and the phosphatase PP-2A [18]. Interestingly, the activity of psychostimulants in the striatum induces a rapid down-regulation of AKT phosphorylation and activity, through a D2-like receptor activity [18]. Importantly, AKT phosphorylation is not down-regulated after psychostimulants treatment in D2R $-/-$ and D2L $-/-$ striata [19], illustrating a specific D2R-mediated effect very likely dependent from activation of D2L.

Future analyses should assess whether the reported effects of D2R-mediated signaling on the AKT and PKA pathways are parallel, and whether they are activated in the same neurons.

D2R-mediated pre-synaptic functions in postsynaptic neurons

Nigrostriatal and mesolimbic afferences, respectively from the SN and VTA, gate sensory, motor and reward information to the striatum. In response to salient events glutamate reward

signals originated in the orbitofrontal cortex and basolateral amygdala reach the ventral striatum where DA is a gatekeeper of these inputs. Similarly, DA modulates glutamate inputs to the dorsal striatum from sensory and motor cortical areas [1], where it filters the noise amplifying the impact of salient stimuli through a D2R-mediated mechanism [20].

In addition to MSNs, D2Rs are also expressed by striatal interneurons [21] with important physiological implications [22,23]. These cells represent only 5% of striatal neurons, however their role is essential in the physiological processing of information relayed from cortical, thalamic and mesencephalic afferences. The participation of cholinergic interneurons on the modulation of MSNs activity, through D2R-dependent signaling has been clearly shown [22, 23]. Presynaptic D2R-mediated mechanisms have also been implicated in the release of GABA and glutamate [20,24,25] from striatal and cortical neurons. Thus, in addition to the DA release modulating function on dopaminergic neurons, D2Rs acting as heteroreceptors, modulate neurotransmitter release from postsynaptic neurons. Thereby the presynaptic release-modulating role of D2Rs influences not only the response of dopaminergic neurons, but also profoundly modify that of target cells.

Presynaptic D2R-mediated function on dopaminergic neurons

Studies on D2R^{-/-} mice have determined that D2 receptors are the “bona fide” autoreceptors regulating DA synthesis and release [26–29]. Interestingly, while the mean baseline concentration of DA in striatal dialysates is similar in WT and D2R^{-/-} siblings, the release of DA evoked by cocaine injection is dramatically higher in D2R^{-/-} mutants as compared to WT animals and well above the range of DA increase normally observed in WT animals [27]. Similar results were also obtained in response to morphine [27].

The observation that D2R-mediated auto-inhibition plays a major role in controlling DA release in conditions of high extracellular DA levels might explain the large influence of D2R on changes induced by drugs of abuse and in particular by cocaine through blockade of the DA transporter (DAT). Thus, in normal conditions D2R autoreceptors, which inhibit firing and DA release, are the only remaining factor able to counteract cocaine effect.

Importantly, selective ablation of the D2L isoform in D2L^{-/-} mice, which still express D2S receptors, does not impair D2R-mediated autoreceptor functions, in support of a specific presynaptic role of the D2S isoform in vivo [8].

Therefore, a deregulation of D2R autoreceptor function, mediated by D2S, might play an important role in the pathophysiology of drug abuse as well as in mediating vulnerability to drugs. This hypothesis is indirectly supported by observations in animals spontaneously vulnerable to drug abuse. These animals are characterized by an enhanced release of DA in response to addictive drugs [30] as well as by a lower number of D2R binding sites [31] and lower inhibition of DA discharge activity resulting from reduced somatodendritic autoreceptor sensitivity [32].

Also, activation of D2Rs has been reported to regulate the trafficking of DAT to the plasma membrane, through activation of the MAPK pathway [33], and that D2Rs physically interact with DAT modulating its activity [34]. Thus, D2Rs, and very likely the D2S isoform, in addition to regulate DA synthesis, strongly participate in the control of its release by different mechanisms among which the interaction with DAT is surely very significant.

The motor stimulating effect of cocaine is impaired by absence of D2S

Largely abused by humans, cocaine elicits its psychomotor and cellular effects by blocking DAT activity on dopaminergic neurons [35]. Glutamate and dopaminergic antagonists abolish

the transcriptional activation of immediate early genes (IEGs) induced by cocaine [36,37]. In this respect, activation of D1Rs is an absolute requirement for the induction of the cellular and behavioral response to cocaine, as demonstrated by studies performed in D1R^{-/-} mice [38]. Recent studies, using transgenic mice in which D1R and D2R containing cells are visualized by the expression of fluorescent proteins, have further refined and supported these findings by showing that the acute cellular response to cocaine mostly engage D1R-, but not D2R-expressing neurons [39].

In this scenario it would be expected that genetic ablation of D2Rs should, if anything, amplify cocaine effects in vivo, due to the reported D2R-dependent inhibitory role on DA signaling. However, this is not what it has been observed.

Cocaine effect on D2R^{-/-} mice has now been evaluated after acute and chronic treatments as well as in self-administration studies with the results that D2R^{-/-} mice have impaired responses to the drug. Importantly, this does not arise from a defective D1R-mediated signaling as the cellular and behavioral responses of D2R^{-/-} mice to direct stimulation of D1Rs is present [40,41]. In line with an unopposed D1R-mediated signaling in D2R^{-/-} mice, activation of the IEG c-fos by D1R-specific agonists at concentrations of D1R ligands that are ineffective to induce the gene in WT mice, resulted into activation of this gene in the striatum of D2R^{-/-} mice [40].

Nonetheless, stimulation of motor activity by cocaine is greatly attenuated in D2R^{-/-} mice with respect to WT controls and it does not increase in a dose-dependent manner [40,42]. Surprisingly, administration of cocaine in D2R^{-/-} mice fails to induce c-fos (Fig.2). This leads to hypothesize that in the absence of D2Rs an inhibitory circuit, normally controlled by D2R, becomes unveiled leading to the reported suppression of c-fos induction in MSNs. GABA and acetylcholine represent good candidates in this context where loss of D2R-mediated control of their release could result into overflows of one or both neurotransmitters [25] on MSNs blocking c-fos induction (Fig.2). Alternatively, loss of D2Rs impairs the formation of macromolecular complexes between the D2R and other proteins, which normally control the cellular and behavioral responsiveness to cocaine [43].

Rewarding and reinforcing properties of addictive drugs in the absence of D2Rs

The rewarding properties of cocaine in D2R^{-/-} mice, as assessed by conditioned place preference (CPP), are attenuated [40]. However, self-administration studies showed that D2R^{-/-} mice self-administer more cocaine than WT mice [44]. The contribution of other neuromodulators (i.e. noradrenalin, serotonin) [45] in expression of CPP and self-administration to cocaine in D2R^{-/-} cannot be excluded and awaits further analyses. This point is of particular relevance in light of the numerous data showing absence of the rewarding effects of several other drugs of abuse in D2R^{-/-} mice. Specifically, D2R^{-/-} mutants are unresponsive to the rewarding and reinforcing properties of morphine [46–48] and alcohol [49,50]. Thus indicating that an intact D2R-mediated signaling is required to elicit the rewarding and reinforcing effects of most drugs.

Importantly, D2L^{-/-} mice, which still express D2S and maintain D2R-mediated autoreceptor functions [8,9,27], have locomotor and rewarding responses to cocaine similar to that of WT animals [40]. Thus implicating a prevalent role of D2S in the behavioral and cellular response to drugs of abuse.

This suggests that presynaptic D2R-mediated effects acting not only on DA release, but also on GABA [25,51,52], glutamate [20] and acetylcholine [22] might play a role in the response to drugs of abuse.

Finally, the specific involvement of D2S and D2L respectively in pre- and postsynaptic activities leaves open the question on the role of the other isoform in either location, since both isoforms are co expressed in D2R expressing neurons. One challenging hypothesis is that trafficking of both isoforms to the membrane might not be equally regulated [53]. The development of the mouse technology and the generation of new animal models and tools should help clarifying this point.

Conclusions

Results obtained from the analysis of D2R mutants have provided evidence of the different involvement of D2L and D2S in D2R-mediated signaling evoked by drugs of abuse and direct agonists. Absence of D2L-mediated signaling impairs the regulation of PKA and AKT pathways by D2Rs, but it does not affect the motor and rewarding response to cocaine. Conversely, D2S-mediated signaling appears to be an absolute requirement for the motor and rewarding effects of cocaine and very likely of other drugs. Future analyses and models are required to further dissect which presynaptic component is involved in these responses, whether that present on dopaminergic or on postsynaptic neurons.

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Figure 1.

Pre- and postsynaptic signaling mediated by D2L and D2S

Schematic representation of D2R-mediated presynaptic and postsynaptic signaling in neurons. At the presynaptic site, D2Rs regulate synthesis and release of dopamine. D2S is the isoform involved in such function as reduced phosphorylation of TH is observed in D2R^{-/-}, but not in D2L^{-/-} mice. Presynaptically, D2Rs also modulate DAT activity, very likely through D2S, through a direct protein-protein interaction.

At the postsynaptic site, D2L mediates G-proteins dependent and independent signaling. The figure shows a simplified scheme of D2R-mediated effects on the PKA and AKT pathways. Activation of D2Rs through the inhibition of adenylyl cyclase decreases DARPP-32 phosphorylation at Thr34. In addition, D2Rs stimulate Ca²⁺-dependent PP-2B activity further increasing dephosphorylation of DARPP-32 at Thr34. Both pathways contribute to a loss of inhibition of the phosphatase activity of PP-1. This signaling is lost in D2R^{-/-} and in D2L^{-/-} mice, pointing to D2L as the D2R isoform involved in postsynaptic signaling.

Activation of D2Rs promotes the formation of a macromolecular complex composed of AKT/PP2-A/ β -arrestin 2. Formation of this complex leads to the inactivation of AKT by PP2-A through dephosphorylation of Thr 308. Inactivation of AKT in response to DA results in the inactivation of GSK3, contributing to the expression of DA-associated behaviors.

Figure 2.

Cellular effects of cocaine on striatal neurons.

Upper panel: Simplified scheme of the effects of acute cocaine treatment in the striatum.

Blockade of DAT by administration of cocaine stimulates DA release, which induces c-fos expression through a glutamate- and D1R-mediated mechanism. D2Rs are also stimulated upon cocaine treatment both pre- and postsynaptically. Presynaptically D2Rs modulate DA release, and postsynaptically they inhibit signaling from MSNs, and regulate glutamate, GABA and ACh release from cortical and striatal neurons.

Lower panel : Administration of cocaine to D2R^{-/-} mice leads to an impressive elevation of extracellular DA in the striatum, due to the loss of D2S-mediated autoreceptor's function.

However, despite an increased activation of D1Rs in D2R^{-/-} mice, c-fos induction is dramatically suppressed. We hypothesize that this is due to loss of regulation of GABA and/or ACh release from striatal neurons and interneurons.

⊥ inhibition; ↓ activation; DA: dopamine/dopaminergic neuron; GABA: gamma-aminobutyric acid/GABAergic Interneuron; ACh: Acetylcholine/Cholinergic Interneuron; Glu: Glutamate/Glutamatergic neuron; DAT: dopamine transporter; D1: dopamine D1 receptor; D2: dopamine D2 receptor; MSN: medium sized spiny neuron; VTA: ventral tegmental area; COC: cocaine.