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Cannabinoid modulation of the dopaminergic circuitry: Implications for limbic and striatal output

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Abstract

Cannabinoid modulation of dopaminergic transmission is suggested by the ability of delta9-tetrahydrocannabinoid to affect motor and motivated behaviors in a manner similar to that produced by pharmacological manipulation of the nigrostriatal and mesocorticolimbic dopamine systems. These behavioral effects as well as analogous effects of endocannabinoids are largely mediated through the cannabinoid type 1 receptor (CB1R). This receptor is located within the substantia nigra and ventral tegmental area, which respectively house the somata of nigrostriatal and mesocorticolimbic dopaminergic neurons. The CB1R is also abundantly expressed in brain regions targeted by the efferent terminals of these dopaminergic neurons. In this review we present the accumulating anatomical and electrophysiological evidence indicating that in each of these systems cannabinoids modulate dopamine transmission largely if not exclusively through indirect mechanisms. The summarized mechanisms include presynaptic release of amino acid transmitters onto midbrain dopamine neurons and onto both cortical and striatal neurons that express dopamine D1-like or D2-like receptors functionally affiliated with the CB1 receptor. The review concludes with a consideration of the psychiatric and neurological implications of cannabinoid modulation of dopamine transmission within these networks.

Keywords

CB1 receptor; D2 receptor; Electron microscopy; Mesocorticolimbic dopamine; Nigrostriatal dopamine

1. Introduction

The rewarding and motor effects produced by endocannabinoids and THC are mediated primarily through the CB1R (Ferre et al., 2010; Monory et al., 2007). CB1Rs and the endocannabinoids 2-AG and anandamide are expressed within the dopamine-enriched basal ganglia networks controlling these behaviors, thus suggesting that the motivational and motor effects of endocannabinoids are ascribed at least in part to modulation of dopamine transmission (Di Marzo et al., 2000; Matyas et al., 2008). The rewarding effects produced by addictive substances as well as food and other natural reinforcers result largely from their capacity to enhance mesolimbic dopamine transmission (Berridge, 2007; Ikemoto, 2010). The key mesolimbic reward pathway originates from dopamine neurons in the VTA that project extensively to the shell compartment of the Acb, but mesocortical dopamine neurons that are also located in the VTA project to the PFC (Lapish et al., 2007; Van den Heuvel and

Pasterkamp, 2008). In contrast, the nigrostriatal dopamine output from the SN to the dorsal striatum is a potent modulator of motor functions (Smith and Villalba, 2008) that are also highly influenced by cannabinoids (Walsh et al., 2010). The involvement of cannabinoids in modulation of dopamine transmission within these neural networks is supported by the abnormalities in reward and motor function in CB1R (–/–) mice (Steiner et al., 1999). In this review, we summarize the evidence indicating that presynaptic and receptor interactive mechanisms enable cannabinoids to fine-tune the activity of mesocorticolimbic and nigrostriatal dopamine neurons (Fig. 1).

2. Microscopic analysis of the endocannabinoid and dopamine systems

Electron microscopic and confocal immunolabeling have contributed significantly to our understanding of the mesocorticolimbic and nigrostriatal dopamine systems and their affiliation with endocannabinoids principally acting through the CB1R. These methods are complementary and also confirm and extend many of the pre- and post-synaptic findings regarding the functionally relevant sites gleaned from electrophysiological methods.

2.1. Electron microscopic imaging

The combined use of electron microscopic immunolabeling and electrophysiological methods led to the initial contention that endocannabinoids acted principally if not exclusively as retrograde signaling molecules in inhibitory-type terminals in highly restricted brain regions (Freund et al., 2003; Katona et al., 1999; Szabo et al., 1998). This idea was based in part on the use of primary and secondary antisera at dilutions that largely detected only those neuronal profiles having high CB1R expression levels such as the GABAergic terminals in the hippocampus (Freund et al., 2003). More widespread but often less robust pre- and post-synaptic CB1R labeling was subsequently shown in many brain regions including those enriched in dopaminergic neurons (Hill et al., 2011; Matyas et al., 2008; Pickel et al., 2004, 2006; Rodriguez et al., 2001). In these regions, electron microscopic and electrophysiological studies concur in showing that the CB1R is an important retrograde signaling molecule in excitatory as well as in inhibitory-type axon terminals (Lupica and Riegel, 2005; Matyas et al., 2008; Robbe et al., 2001). The striking affiliation between the CB1R and the postsynaptic density of excitatory-type synapses was similarly first demonstrated by electron microscopic immunolabeling in striatum (Rodriguez et al., 2001) and subsequently confirmed in other brain regions (Nyiri et al., 2005).

The electron microscopic demonstration of 2-AG in postsynaptic dendrites and dendritic spines contacted by CB1R-containing axon terminals strongly supports the idea that lipid-derived endocannabinoids are prominently involved in retrograde signaling (Katona et al., 2006; Nyilas et al., 2008; Yoshida et al., 2006). In contrast, NAPE-PLD, a biosynthetic enzyme of the endocannabinoid anandamide, is predominantly localized presynaptically in axon terminals inclusive of those forming axodendritic excitatory-type synapses and those apposed to other axons and axon terminals. This suggests that endocannabinoids modulate the release of neurotransmitters through mechanisms other than retrograde signaling. These mechanisms may include communication between neighboring axons and from presynaptic terminals to postsynaptic dendrites. The diverse CB1R-mediated effects of endocannabinoids may be particularly important in brain regions receptive to dopaminergic terminals that are frequently apposed to excitatory-type terminals convergent on single dendritic spines as in the striatum (see below).

2.2. Detailed immunolabeling protocol for electron microscopy

The electron microscopic images in this review were obtained from male C57BL/6J mice (20–25 g; Jackson Laboratory, Bar Harbor, ME) using experimental procedures in

agreement with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals, and approved by the Institutional Animal Care and Use Committees (IACUC) at Weill Medical College of Cornell University. All efforts were used to reduce the amount of animals needed and to minimize their suffering. To achieve this, the mice were deeply anesthetized by intraperitoneal injection of sodium pentobarbital (150 mg/kg). Brain tissue was then fixed by vascular perfused with 30 ml of a solution containing 3.75% acrolein and 2.0% paraformaldehyde in 0.1 M phosphate buffer, (PB; pH 7.4) followed by 150 ml of 2% paraformaldehyde in PB. After removal from the cranium, the brains were post-fixed for 30 min in 2% paraformaldehyde in PB and cut into 40 μ m coronal sections that included the VTA, SN and striatum as identified in the mouse atlas (Franklin and Paxinos, 1997). These tissue sections were collected in 0.1 M PB and then placed for 30 min in a solution of 1% sodium borohydride in 0.1 M PB to remove excess active aldehydes prior to processing for immunolabeling. For this, we used antisera specifically recognizing the CB1R and tyrosine hydroxylase (TH), a rate-limiting catecholamine synthesizing enzyme highly expressed in dopamine neurons (Augood et al., 1993; Lorang et al., 1994). The guinea pig polyclonal CB1R antiserum was raised against the full C-terminus of the rat CB1R and generously provided by Dr. Ken Mackie, Department of Psychological & Brain Sciences, MSBII 120, Indiana University, Bloomington, IN. The monoclonal mouse antibody was raised against an epitope within the mid-portion of TH (ImmunoStar, Hudson, WI). The CB1R and TH antisera were used to respectively identify the corresponding antigens in single sections of tissue using immunoperoxidase and immunogold silver methods initially developed by Chan et al., 1990 and used extensively with minor modifications for detection of many different antigens in brain (Lane et al., 2010b; Nirenberg et al., 1997; Pickel et al., 2006; Van Bockstaele et al., 1996). The immunolabeling protocol was initiated by an overnight incubation of tissue sections at room temperature in a mixture of the guinea pig anti-CB1R antiserum (1:3000) and the mouse anti-TH antiserum (1:25,000) in a solution of Tris-saline containing 0.1% bovine serum albumin (BSA; Sigma-Aldrich, St. Louis, MO). These sections were then washed and placed for 30 min in biotinylated donkey anti-guinea pig immunoglobulin (IgG, 1:200) (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) followed by a 30 min incubation in Vectastain ABC Elite kit (Vector Laboratories, Burlingame, CA). The product was visualized by reaction in 3,3'-diaminobenzadine (DAB, Sigma-Aldrich, St. Louis, MO) and hydrogen peroxide. Subsequently, for immunogold labeling of the mouse TH antiserum, the tissue was washed and placed in a solution of Ultrasmall gold (Electron Microscopy Sciences, Hatfield, PA) conjugated to donkey anti-rabbit IgG. The particles were visualized by using the Silver Intense kit (GE Healthcare). The immunolabeled sections of tissue were post-fixed in 2% osmium tetroxide and embedded in plastic to enable the collection of ultrathin sections from the surface of the tissue with a Leica ultramicrotome (Leica Microsystems, Wetzlar, Germany). These ultrathin sections were counterstained on mesh grids using uranyl acetate and lead citrate prior to examination with a FEI CM-10 electron microscope (FEI, Hillsboro, OR).

2.3. Fluorescent confocal imaging

The use of confocal fluorescent microscopy has elucidated the distribution of the CB1R among different cell types in the rodent brain. While confocal fluorescent microscopy lacks the subcellular resolution of electron microscopy, it is the more appropriate methodological technique to answer hypotheses regarding the gross localization and/or colocalization of two or more proteins; for example, the question of which GABAergic cell type(s) express the CB1R in various brain regions using markers for different interneurons. Fluorescent confocal microscopy has been used to demonstrate a high degree of expression of the CB1R within interneurons expressing CCK in the cortex but little to no expression of the CB1R by fast-firing parvalbumin interneurons (Bodor et al., 2005). Interestingly, this lack of

expression of the CB1R by parvalbumin-positive interneurons is not consistent throughout the rodent brain. In the striatum, large parvalbumin interneurons frequently contain the CB1R as do a large proportion of GABAergic medium spiny projection neurons (Fusco et al., 2004).

Furthermore, the various fluorophores and lasers available for confocal microscopy allow concurrent visualization of more than two receptors and/or enzymes, while electron microscopy is limited to two secondary markers — immunoperoxidase and immunogold. For example, in the PFC, TH is contained in both dopaminergic projections from the VTA as well as noradrenergic circuitry (Miner et al., 2003). Therefore, to determine whether the CB1R is localized to dopaminergic axon terminals in the PFC, it is necessary to use confocal microscopy and triple immunolabel for the CB1R, TH, and the norepinephrine transporter (NET) expressed by noradrenergic axonal processes in this brain region (Miner et al., 2003).

2.4. Detailed triple-labeling methods for fluorescent immunohistochemistry

Mouse brain tissue was fixed by vascular perfusion with 4% paraformaldehyde. The aldehyde-fixed mouse brains were cut into 40 μ m coronal sections on a Vibratome (Leica, Deerfield, IL), and sections through the PFC were selected at 1.94 mm anterior to Bregma for processing. These sections of tissue were rinsed in 0.05 M phosphate buffered saline (PBS; pH 7.4) prior to blocking in 0.25% Triton (Sigma-Aldrich, St. Louis, MO) with 0.5% BSA and 5% normal goat serum (NGS; Jackson ImmunoResearch, West Grove, PA) in 0.05 M PBS for 2 hours. Brain sections were then washed in 0.05 M PBS prior to overnight incubation at room temperature in the primary antibody cocktail containing 0.1% Triton, 0.5% BSA, and 5% NGS with primary antisera. The CB1R was labeled using an affinity-purified antibody raised in guinea pig, generously provided by Dr. Kenneth Mackie, at a 1:1500 dilution. TH was identified using a mouse monoclonal antibody commercially available through Incstar (Stillwater, MN) at a 1:10,000 dilution. The NET was probed using an affinity-purified antibody raised in rabbit at a 1:5000 dilution and generously provided by Dr. Randy D Blakely, Department of Pharmacology and Psychiatry, Vanderbilt University, Nashville, TN, USA. The tissue was placed subsequently in 0.1% Triton and 5% NGS in PBS for 30 min over multiple washes and rinsed in PBS. Tissue was incubated for two hours in 0.1% Triton and 5% NGS in PBS containing secondary antibodies goat anti-guinea pig AlexaFluor488 (1:400; Invitrogen, Carlsbad, CA), goat anti-rabbit AlexaFluor633 (1:400), and goat anti-mouse AlexaFluor546 (1:400). Brain sections were washed for one hour over multiple rinses in PBS prior to mounting on subbed slides in 0.05 M PB, coverslipped with ProLong Gold antifade mounting media (Invitrogen), and sealed with clear nail polish.

Slides were imaged using a Leica TCS SP5 confocal microscope interfaced with LAS AF Version 2.0.2 (Leica Microsystems, © 2005–2008) computer software on a PC computer. Images of individual channels were sequentially captured using a monochrome CCD camera and were pseudocolored using the LAS AF software. Images were digitally collected in a 1024 $\times$ 1024 pixel frame using $\times$ 20, $\times$ 40, or $\times$ 63 objective lenses. Images from individual channels and the compilation of all three channels are shown. Adobe Photoshop CS4 (Adobe Systems, Inc.) was used to compile images, crop, adjust brightness and contrast, incorporate symbols and text, and prepare figures.

3. Cannabinoid modulation of midbrain dopamine neurons

Endocannabinoid activation of CB1 receptors can potently modulate the activity of midbrain dopaminergic neurons located within the VTA or SN. The endocannabinoid mechanism of action in these brain regions is the same as in retrograde signaling in glutamatergic afferents derived from the PFC and the subthalamic nucleus (Morera-Herreras et al., 2008; Pan et al., 2008).

3.1. Ventral tegmental area

Enhanced excitability and bursting of dopamine neurons in the VTA promotes the release of endocannabinoids that activate presynaptic Gi-coupled CB1Rs in both GABAergic and glutamatergic terminals (Riegel and Lupica, 2004). CB1R-mediated inhibition of GABA and glutamate release results respectively in depression and facilitation of bursting activity in mesocorticolimbic dopaminergic neurons of the VTA (Riegel and Lupica, 2004). This physiological evidence is supported by electron microscopic detection of CB1Rs in axon terminals that form either symmetric (inhibitory-type) or asymmetric (excitatory-type) synapses with VTA dendrites that contain TH immunoreactivity (Fig. 2; see also Matyas et al., 2008). Furthermore, cannabinoid withdrawal after chronic administration produces a selective hypofunction of dopamine neurons in the VTA (Spiga et al., 2010), which is consistent with the schematized location of presynaptic CB1Rs shown in Fig. 1B.

3.2. Substantia nigra

CB1R immunolabeling is also abundant in the SN, but this labeling is principally located in the SN pars reticulata (SNpr), a region containing mainly GABAergic neurons and distinct from the pars compacta (SNpc) that contains the nigrostriatal dopaminergic neurons (Glass et al., 1997; Walsh et al., 2010). Combined in situ hybridization for CB1mRNA and CB1R immunoreactivity indicates that CB1Rs in the SN are synthesized in striatal somata and transported through the direct striatal output pathway to their terminals in the SNpr (Julian et al., 2003). In this region the activation of CB1Rs can inhibit the release of GABA from striatonigral terminals providing input to the postsynaptic neurons that also contain GABA (Wallmichrath and Szabo, 2002). In this region, the CB1R is also expressed in many excitatory-type axon terminals (Szabo et al., 2000).

Dendrites of dopaminergic neurons in the SNpc extend into the SNpr, and some of these dendrites receive input from CB1R-containing axon terminals. These terminals frequently form symmetric synapses, and more rarely form asymmetric synapses, with TH-containing dopaminergic dendrites (Fig. 3). The potentiation of GABAergic inhibition of nigrostriatal dopaminergic neurons is thought to play an important role in cannabinoid-induced motor inhibition (Romero et al., 1998), an effect resembling that seen in patients and in animal models of Parkinson's disease (Day et al., 2008). In addition, however, the presence of the CB1R in some of the axon terminals that form asymmetric excitatory-type synapses on TH-containing dendrites in the SN indicates a possible pathway-specific involvement of endocannabinoids in modulating glutamate-induced depolarization of dopamine neurons in the SN (Melis et al., 2000).

4. Cannabinoid and dopamine convergence in dorsal striatum

Dorsal striatal dopamine terminals identified by the presence of TH and/or the DAT principally form punctate symmetric or non-synaptic contacts with dendritic spines (Nirenberg et al., 1996; Pickel et al., 1996). CB1Rs are found in a separate population of small axons and axon terminals in proximity to dopaminergic terminals defined by TH immunoreactivity (Fig. 4). Of several hundred axonal profiles containing CB1R immunoperoxidase labeling that we have observed in mouse striatum, only two, one of which is shown in Fig. 4B, contained a single TH immunogold particle. This suggests that there is little if any expression of the CB1R in axon terminals of nigrostriatal dopaminergic neurons. Thus, the more indirect mechanisms diagrammatically shown in Fig. 1C are likely to play a major role in the potent cannabinoid modulation of motor functions under the control of nigrostriatal dopaminergic neurons.

Dorsal striatal neurons are part of a neural network that dynamically adjusts its responses in flux with extrinsic environmental factors through dopaminergic transmission (Boehler et al.,

2011). Dopamine activates D1- and/or D2-like families of dopamine receptors in medium spiny striatal projection neurons, which comprise the over-whelming majority of neurons in this region (Di Marzo et al., 2000). In dorsal striatal projection neurons, the functionally opposing dopamine D1 and D2 receptors are respectively segregated into striatonigral (direct) and striatopallidal (indirect) pathways (see Lopez de Maturana and Sanchez-Pernaute, 2010; Yung and Bolam, 2000 for extensive review). Endocannabinoids not only contribute to D2R-mediated inhibition, but also to D1R-mediated enhancement of the frequency of spontaneous excitatory postsynaptic currents in the subpopulations of medium spiny striatal neurons targeted by dopamine (Andre et al., 2010). The D1R-containing neurons are inclusive of those that express dynorphin and participate in the motor-depressant effects under the influence of presynaptic CB1Rs while D2R interaction with postsynaptic CB1Rs are thought to largely mediate the cataleptogenic effects of cannabinoids (Ferre et al., 2010).

The motor effects produced by acute CB1R agonist administration are similar to those produced by antagonists of the D2R (Svensson, 2000), thereby suggesting functional antagonism between the cannabinoids and dopamine in the dorsal striatum. Glutamatergic striatal terminals, many of which are derived from neurons in the cerebral cortex (Bouyer et al., 1984) express D2Rs (Wang and Pickel, 2002). These terminals often form asymmetric synapses on dendritic spines, as do many of those containing the CB1R in the dorsal striatum (Matyas et al., 2006; Rodriguez et al., 2001). In addition to their shared presynaptic location, the Gi-coupled CB1 and dopamine D2 receptors are also present in many of the same postsynaptic striatal neurons in which their dual in vitro activation elicits an unexpected stimulatory effect on cAMP (Glass and Felder, 1997; Kearn et al., 2005). The formation of functionally opposed CB1/D2 receptor heterodimers in the large population of medium spiny projection neurons in striatum (Fig. 1C) may provide a powerful mechanism by which cannabinoids can alter D2R-mediated inhibition of striatal output neurons.

Functional antagonism between CB1 and D2 receptors may also exist in striatal aspiny interneurons, which are much fewer in number but highly influential on the activity of the output neurons in dorsal striatum (Bennett and Bolam, 1994). In this region, endocannabinoids have been shown in paired recordings to retrogradely inhibit GABA release from terminals of fast-spiking interneurons presynaptic to medium spiny neurons (Narushima et al., 2006). Striatal fast-spiking interneurons contain parvalbumin, a calcium-binding protein, and frequently express the CB1R (Fusco et al., 2004). The level of parvalbumin expression in striatal interneurons is significantly reduced in CB1 (–/–) mice (Fitzgerald et al., 2011), which may be indicative of decreased firing activity of this population. These striatal interneurons receive excitatory input from the cerebral cortex and provide inhibitory input to medium spiny neurons that project to the SN (Bennett and Bolam, 1994). Dopamine signaling contributes to the plasticity of glutamatergic corticostriatal transmission (Lopez de Maturana and Sanchez-Pernaute, 2010). Endocannabinoid signaling is particularly critical for the induction of long-term depression at both glutamatergic and GABAergic synapses (Adermark and Lovinger, 2007; Adermark et al., 2009). This suggests the involvement and integration of endocannabinoid and dopamine signaling in feed-forward inhibition of striatal output.

5. Cannabinoid involvement in mesocorticolimbic dopamine transmission

The mesocorticolimbic dopamine system allows internal drives and environmental contingencies to guide goal-directed behavior so that ongoing activities are maintained by evoked phasic dopamine release (Sesack and Grace, 2010). Conditioned cue-induced tonic increases in mesolimbic dopamine are believed to largely result from downstream stimulation of midbrain dopamine neurons through glutamatergic afferents arising in the

prefrontal cortex and/or amygdala (Volkow et al., 2008). Behavioral effects of cannabinoids are likewise context-dependent and cue-induced suggesting that some of these effects may involve functional interactions with the mesocorticolimbic dopamine system (Haller et al., 2004; Mikics et al., 2006). The relevant interactive connections include convergence between dopaminergic terminals and glutamatergic inputs from the PFC onto dendritic spines in the Acb, a region critically involved in reward circuitry (Sesack and Pickel, 1992). In this region, the activation of dopamine D2 receptors can pre-synaptically modulate glutamate release (Kalivas and Duffy, 1997) in a manner that is crucial for instrumental response selection in the context of reward as well as in psychostimulant-induced euphoria (Koch et al., 2000).

5.1. Mesolimbic dopamine

CB1Rs are prevalent in inhibitory as well as in some excitatory-type terminals within limbic brain structures including the hippocampus, amygdala, Acb, and the VTA, where the somata of mesolimbic and mesocortical dopaminergic neurons are located (Bodor et al., 2005; Freund et al., 2003; Katona et al., 2001). As previously indicated, the CB1R is located in axon terminals forming either inhibitory-or excitatory-type synapses with dopaminergic as well as non-dopaminergic putative GABAergic neurons in the VTA (Matyas et al., 2008). This electron microscopic immunocytochemical data is consistent with electrophysiological evidence that activation of CB1Rs in the VTA suppresses depolarization-induced excitation (DSE) of glutamatergic inputs from the prefrontal cortex as well as inhibition (DSI), the two most well characterized forms of endocannabinoid-mediated synaptic plasticity (Melis et al., 2004; Riegel and Lupica, 2004). Moreover, systemic administration of a CB1R agonist enhances the bursting activity of VTA dopamine neurons, many of which project to the Acb, where there is a concomitant fluctuation in extracellular dopamine (Cheer et al., 2004). This effect may reflect either local presynaptic mechanisms in the VTA or the Acb, a portion of the ventral striatum that consists of shell and core compartments (Hoffman and Lupica, 2001). The functional distinction between these compartments reflects in part the more extensive input to the Acb shell from the VTA and to the Acb core from the substantia nigra (Heimer et al., 1997; Zahm, 2000; Zahm et al., 1996). The Acb shell also receives substantial prefrontal cortical and subcortical inputs from limbic brain regions including the VTA. These different afferents converge on many of the same medium spiny neurons (Sesack and Pickel, 1990, 1992). Mice lacking the CB1R show a reduction in TH-labeled varicosities, the majority of which are dopaminergic, in the Acb shell as well as a loss of dopamine-dependent reward function (Lane et al., 2010a).

The anatomical relationship between neurons expressing the CB1R and dopamine in the VTA and Acb is analogous to that observed in the SN and dorsal striatal circuitry. As in the dorsal striatum, many of the Acb neurons also co-express CB1 and D2 receptors (Pickel et al., 2006). Thus, they are potential sites where cannabinoids may significantly modulate dopamine transmission through intracellular heterodimerization following dual activation of these receptors (see above discussion in dorsal striatum). In addition to postsynaptic co-localization in Acb neurons, D2 and CB1 receptors are distributed in many of the same as well as separate axonal profiles including small axons and axon terminals (Pickel et al., 2006). In the Acb, about 20% of CB1R-containing terminals contact dendrites that express the D2R, forming either asymmetric (excitatory-type) or symmetric (inhibitory-type) synapses (Pickel et al., 2006). These results suggest that cannabinoid modulation of presynaptic release of glutamate onto neurons expressing Gi-coupled D2Rs may facilitate D2R-mediated inhibition, while CB1R activation in GABAergic terminals may oppose this inhibition.

5.2. Mesocortical dopamine

Ultrastructural studies combining track tracing with electron microscopic immunolabeling have provided evidence for a synaptic circuitry in which mesolimbic dopamine transmission is modulated by excitatory outputs from the PFC that target dopamine dendrites in the VTA and converge with dopamine terminals in the Acb (Sesack and Pickel, 1992). Furthermore, in vitro electrophysiological studies indicate that the activity of dopaminergic neurons in the VTA is controlled in part by activation of presynaptic CB1Rs found in glutamatergic terminals derived from the PFC (Lupica and Riegel, 2005). The PFC also provides extensive glutamatergic input to GABAergic neurons of the VTA, which in turn modulate dopaminergic neurons (Carr and Sesack, 2000a, b). Thus, the activation of CB1Rs provides an important mechanism for indirectly modulating dopamine transmission through actions on PFC glutamatergic outputs to the VTA and Acb.

Within the cortex, the CB1R is primarily expressed on presynaptic axon terminals of GABAergic basket interneurons, most of which also contain CCK (Bodor et al., 2005). In deep layers of the PFC, these CB1R-containing axon terminals frequently form symmetric inhibitory-type synaptic contacts on the soma of pyramidal projection neurons and CB1R activation induces increased functional output of these neurons (Hill et al., 2011). Many of these same CB1R-containing inhibitory presynaptic terminals in the PFC also contain the D2R, and dopamine activation of the D2R may facilitate the actions of endocannabinoids in this region (Chiu et al., 2010). Activation of presynaptic CB1Rs on GABAergic terminals in the PFC induces endocannabinoid-mediated inhibitory long-term depression (iLTD) and concurrent stimulation of the D2R suppresses GABA release, promoting this iLTD in the PFC (Chiu et al., 2010). This direct synaptic interaction of the D2R and the CB1R regulates cortical inhibitory tone which may be altered in neuropsychiatric illnesses such as schizophrenia (Lewis et al., 2005).

While TH-labeled axonal varicosities in the PFC have not been observed to express the CB1R, axons differentially expressing TH and CB1R frequently share the same postsynaptic target (Fig. 5). This suggests that the dopamine terminals converge on single neurons with axon terminals containing neurotransmitters, whose release is controlled in part through CB1R-mediated retrograde signaling (Fig. 1). The CB1R was not only absent from TH-immunolabeled processes, but also from those processes that were dually labeled for TH and the NET (Fig. 5). The majority of axons containing the NET were at least lightly labeled for TH somewhere along their length. CB1R-, TH-, and TH/NET-immunoreactive axonal processes frequently surrounded unlabeled soma (white arrow, Fig. 5). This may indicate that a postsynaptic neuron receptive to dopaminergic innervation is involved in endocannabinoid signaling, and implies a convergence of dopaminergic and cannabinoid signaling pathways within the same postsynaptic cell. In fact, in vivo evidence of this exists in the striatum, where dopamine activation of the D2 receptor combined with postsynaptic depolarization induces the release of endocannabinoids (Yin and Lovinger, 2006). Furthermore, post-synaptic targets of CB1R-containing axon terminals often express the dopamine D2R in the mPFC (unpublished observations). These postsynaptic D2R-expressing targets of CB1R-containing axon terminals include many somata and dendrites of pyramidal projection neurons and more rarely include parvalbumin interneurons. Interestingly, light microscopic immunolabeling studies reveal a significant decrease in expression of the calcium binding protein parvalbumin in fast-spiking interneurons of the mPFC in CB1R KO mice (Fitzgerald et al., 2011). These cortical parvalbumin interneurons contain D2 but not CB1 receptors. Furthermore, maturation of parvalbumin expression in the cortex is dependent upon D2R activation (Porter et al., 1999), suggesting that the observed decrease in parvalbumin may be induced by altered dopamine signaling in CB1R KO mice. Together, these observations indicate that the CB1R-mediated endocannabinoid system is a critical factor in mesocorticolimbic dopamine signaling, and vice versa.

6. Implications

This review summarizes the substantial evidence that cannabinoids modulate dopamine transmission in both the nigrostriatal and mesocorticolimbic systems by indirect means involving CB1R-mediated inhibition of amino acid transmission and/or convergent activation of CB1 and dopamine receptors (Fig. 5). This modulation is of direct relevance for complex behaviors that are impaired in many neurological and psychiatric disorders including drug addiction (Lopez-Moreno et al., 2008). Thus, the findings have important implications for understanding the etiology of a variety of clinically relevant diseases and may contribute to developing new avenues of treatment, conceivably through drugs targeting CB1/D2 receptor heteromers (Ferre et al., 2010; Wang et al., 2000). Whether or not such treatments would be equally applicable to both men and women remains to be determined, since much of the existing knowledge of cannabinoid–dopamine interactions is based on studies of male rodents and estrogen has been shown to modulate THC-induced changes in mesolimbic dopamine transmission (Bonnin et al., 1993).

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Abbreviations

2-AG	2-arachidonoylglycerol
Acb	nucleus accumbens
CB1R	cannabinoid CB1 receptor
CCK	cholecystokinin
D1R	dopamine D1 receptor
D2R	dopamine D2 receptor
DAT	dopamine transporter
GABA	gamma-Aminobutyric acid
NAPE-PLD	N-acylphosphatidylethanolamine-hydrolyzing phospholipase D
NET	norepinephrine transporter
PFC	prefrontal cortex
SN	substantia nigra
SNpc	substantia nigra pars compacta
SNpr	substantia nigra pars reticulata
TH	tyrosine hydroxylase
THC	delta9-tetrahydrocannabinoid
VTA	ventral tegmental area

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Fig. 1.

Schematic diagram illustrating the key sites for cannabinoid modulation of dopamine reward- and motor-associated pathways of the rodent brain discussed in this review. (A) Dopaminergic projections (purple) from the VTA and the SN target postsynaptic neurons in the Acb shell and core (blue), the dorsal striatum/caudate putamen (CPu; blue), and the PFC (yellow). (B) In both the VTA and SN, glutamatergic and GABAergic axon terminals expressing the CB1R target TH-containing dopaminergic projection neurons (observations reported herein; Matyas et al., 2008). (C) In both the CPu and Acb, CB1Rs are expressed within medium spiny neurons, the most predominant cell type in these regions (Pickel et al., 2006; Rodriguez et al., 2001). These receptors are also co-localized with the D2R in dendritic shafts and dendritic spines of these striatal neurons. Both glutamatergic and

GABAergic axon terminals contain the CB1R in the striatum. Glutamatergic terminals expressing the CB1R occasionally contact dendritic spines containing the D2R. (D) In the PFC, the illustrated pyramidal neuron is targeted by GABAergic axon terminals (blue) which contain the CB1R (yellow stars). In this region, the CB1R is a presynaptic receptor that is expressed in many GABAergic inhibitory-type axon terminals but also present within glutamatergic excitatory-type axon terminals (red) (Lafourcade et al., 2007). Many of these same pyramidal neurons are also targeted by dopaminergic terminals (Carr et al., 1999). In the PFC, the D2R (black stars) is located within post-synaptic dendrites as well as in axon terminals, many of which also contain the CB1R (Chiu et al., 2010).

Fig. 2.

Electron micrographs through the mouse VTA show synaptic contacts between axon terminals containing the CB1R and dendrites labeled for TH. In (A), immunoperoxidase labeling for the CB1R is seen in an axon terminal (CB1R-te) that forms an inhibitory-type symmetric synapse (black arrowhead) with a dendritic profile containing many TH-immunogold-silver deposits (TH-de). CB1R immunoperoxidase is most abundant near the presynaptic density, and is evident in contrast with an unlabeled terminal (u-te). Likewise, in (B), a TH-immunogold labeled dendrite is contacted by an axon terminal containing CB1R immunoperoxidase. This terminal forms a junction synapse with a slightly thickened postsynaptic membrane specialization typical of asymmetric synapses (white arrowhead). Scale bars = 500 nm.

Fig. 3.

Electron microscopic images of mouse substantia nigra (SN) show that in this brain region, CB1R-immunoperoxidase labeled axon terminals contact TH-immunoreactive dendrites. (A) An axon containing CB1R-immunolabeling (CB1R-a) opposes a dendritic profile with TH-immunogold labeling (TH-de). (B) An axon terminal containing CB1R immunoperoxidase labeling (CB1R-te) forms an asymmetric excitatory-type synaptic contact (white arrowhead) with a dendrite containing TH immunogold-silver particles. Scale bars = 500 nm.

Fig. 4.

In the mouse striatum, CB1Rs and TH are primarily localized in disparate compartments of the neuropil as seen by electron microscopic immunolabeling. In (A), CB1R-immunoperoxidase labeled axonal profiles (CB1R-a) are seen within neuropil that also contains axon terminals expressing TH (TH-t), as indicated by the presence of immunogold particles overlying small synaptic vesicles. (B) A CB1R-immunoperoxidase reactive axon terminal (CB1R-t) forms a symmetric inhibitory-type synaptic contact (black arrowhead) with an unlabeled dendrite (U-de). Multiple TH-immunogold labeled axons (TH-a) and terminals (TH-t) are in the surrounding neuropil. A single axon is dual labeled for TH immunogold and CB1R immunoperoxidase (black star). Scale bars = 500 nm.

Fig. 5.

Confocal images of the mouse PFC show many CB1R-labeled axonal varicosities that also express the NET but not TH, a catecholamine synthesizing enzyme that is expressed in substantially higher levels in dopaminergic compared with noradrenergic neurons. NET immunolabeled processes are shown in the blue channel (A), TH-labeled axonal processes in the red channel (B) and CB1R-labeled processes in the green channel (C). In (D), the images are merged to show CB1R, TH, and NET immunolabeling. The NET is frequently found within TH-labeled processes, but neither is observed to colocalize with the CB1R. Labeled axon varicosities form circular patterns in the neuropil, an arrangement of labeling consistent with the idea that synaptic boutons separately expressing the CB1R and TH/NET may surround and synapse onto unlabeled neuronal somata. The white arrow indicates one such putative unlabeled neuronal soma contacted by varicose axons that are separately labeled for CB1 and TH/NET, thereby suggesting convergent cannabinoid and dopamine targeting. Scale bars = 25 μ m.