Cannabinoid–Dopamine Interaction in the Pathophysiology and Treatment of CNS Disorders

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Endocannabinoids and their receptors, mainly the CB_1 receptor type, function as a retrograde signaling system in many synapses within the CNS, particularly in GABAergic and glutamatergic synapses. They also play a modulatory function on dopamine (DA) transmission, although CB_1 receptors do not appear to be located in dopaminergic terminals, at least in the major brain regions receiving dopaminergic innervation, e.g., the caudate-putamen and the nucleus accumbens/prefrontal cortex. Therefore, the effects of cannabinoids on DA transmission and DA-related behaviors are generally indirect and exerted through the modulation of GABA and glutamate inputs received by dopaminergic neurons. Recent evidence suggest, however, that certain eicosanoid-derived cannabinoids may directly activate $TRPV_1$ receptors, which have been found in some dopaminergic pathways, thus allowing a direct regulation of DA function. Through this direct mechanism or through indirect mechanisms involving GABA or glutamate neurons, cannabinoids may interact with DA transmission in the CNS and this has an important influence in various DA-related neurobiological processes (e.g., control of movement, motivation/reward) and, particularly, on different pathologies affecting these processes like basal ganglia disorders, schizophrenia, and drug addiction. The present review will address the current literature supporting these cannabinoid-DA interactions, with emphasis in aspects dealing with the neurochemical, physiological, and pharmacological/therapeutic bases of these interactions.

The Control of Neurotransmitter Activity by Endocannabinoids

Endocannabinoids and their receptors play a modulatory function in several physiological processes, mainly in the brain [1–3] but also in various peripheral processes such as the immune regulation [4], the cardiovascular system [5], the reproductive endocrine processes [6], and the control of energetic metabolism [7]. In the brain, endocannabinoids participate in processes such as the control of movement [8–10], nociception [11], brain reward [12], learning and memory [13], feeding [14], and emesis [15], as well as they play an important role in various events related to brain development [16,17]. This large list of brain functions, in which endocannabi-

noids and their receptors are active, is the result of numerous studies developed during the last 20 years which demonstrated that: (i) changes in these processes are included within the spectrum of pharmacological actions in humans and laboratory animals of those compounds capable to activate or to inhibit the cannabinoid system (reviewed in [18–20]); (ii) cannabinoid receptors, mainly the CB_1 receptor type, as well as their endogenous ligands, mainly anandamide and 2-arachidonoylglycerol, are abundant in the brain structures involved in the above processes [2,21]; and/or (iii) mice lacking specific genes of the cannabinoid signaling such as those encoding for the CB_1 or CB_2 receptor, or for the enzymes N-arachidonoyl-phosphatidylethanolamine (NAPE-PLD) or fatty acid amide hydrolase (FAAH), exhibited
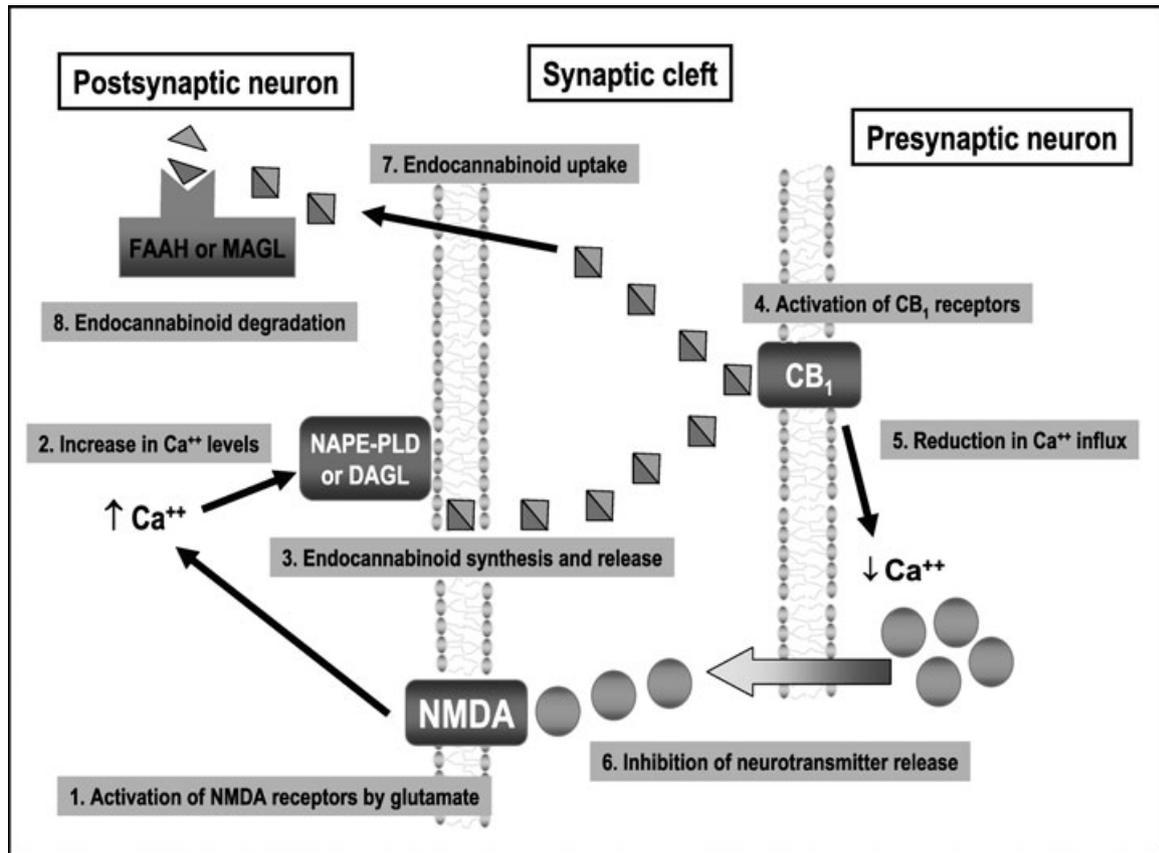
Figure 1 Processes involved in the function of endocannabinoids and CB$_1$ receptors as a retrograde signaling mechanism in glutamatergic neurons.

behavioral changes compatible with a role of the cannabinoid system in such processes [22–24]. An important consequence of these brain functions proposed for the cannabinoid signaling system is that it may be considered of therapeutic relevance for different pathologies related to these brain functions [20,25], which explains the increasing development in this field during the last years.

The involvement of the endocannabinoid system in this large list of brain functions is likely the consequence of its capability to interact with specific neurotransmitters in several brain regions [26]. For many years, most of researchers tried to demonstrate that endocannabinoids and their receptors may function as a novel transmitter system mimicking the process developed in the 1970s with the discovery of the opioid system. However, the action of endocannabinoids in the synaptic function appeared to be more compatible with a modulatory role rather than with a function as a classic transmitter. For example, the frequent, although not exclusive, presynaptic location of CB$_1$ receptors allows endocannabinoids to directly influence presynaptic events, such as synthesis, release or reuptake, for specific transmitters, mainly glutamate, opioid peptides and GABA, since CB$_1$

receptors are frequently located onto neurons containing these neurotransmitters in the brain, but also for acetylcholine and serotonin (for review, see [27]). The combination of numerous pharmacological, electrophysiological and immunohistochemical studies allowed to demonstrate that endocannabinoids function as retrograde signal molecules at the synapse (for review, see [1,3], and an overview in Figure 1), in particular in glutamatergic and GABAergic synapses, then preventing an excess of excitation or inhibition, respectively [28].

Dopamine (DA) is one of the neurotransmitters that has been more frequently linked to the action of cannabinoids within the CNS. This can be applied to the case of those dopaminergic neuronal subpopulations, whose cell bodies are located in the reticular formation of the midbrain (e.g., substantia nigra and ventral-tegmental area), and that project to different forebrain structures, namely, the caudate-putamen (nigrostriatal pathway; see Figure 2), and the nucleus accumbens/prefrontal cortex complex (mesocorticolimbic pathway). Both neuronal systems would exert a regulatory action on different effector neurons in these structures, then influencing processes such as the control of movement and various
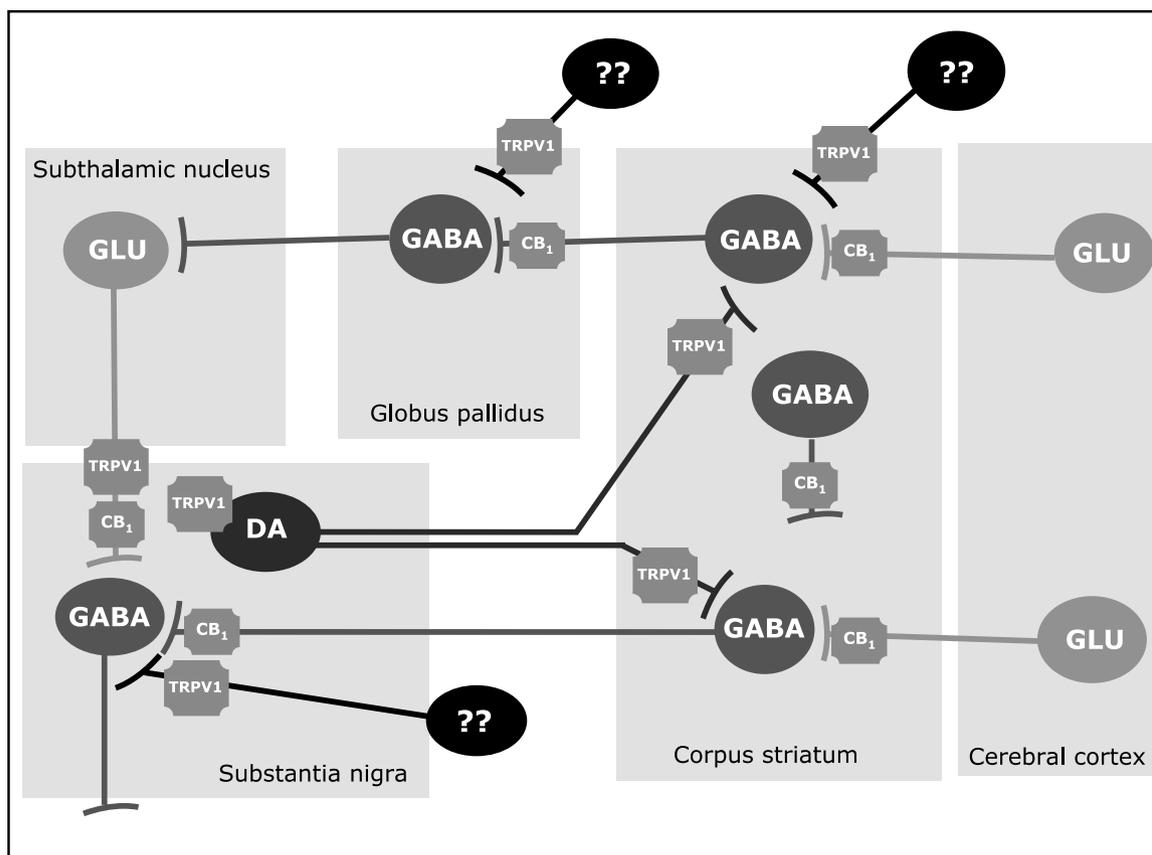
Figure 2 Distribution of CB_1 and $TRPV_1$ receptors in different neuronal subpopulations in the basal ganglia circuitry (DA, dopamine; GABA, γ-aminobutiric acid; GLU, glutamate).

cognitive functions, respectively, effects that are among the most relevant pharmacological actions of cannabinoids [8–10,12]. It is also important to remark that deficiency or overactivity of these dopaminergic pathways can result in disorders, such as Parkinson's disease (PD) and schizophrenia, respectively, for which various cannabinoid-related compounds have been proposed as a novel type of therapy [10,29]. However, despite this close pharmacological interaction, there is little evidence supporting that dopaminergic neurons in the basal ganglia and limbic structures contain CB_1 receptors [30,31]. Most of the researchers agree that cannabinoid effects on DA transmission are frequently indirect and exerted by either postsynaptic or presynaptic mechanisms (reviewed in [32,33]). For these authors, the abundance of CB_1 receptors in GABAergic, glutamatergic or opioidergic projections located in the closest vicinity of dopaminergic neurons [34–36], facilitates such indirect action. This is also supported by data showing that midbrain dopaminergic neurons, although do not contain CB_1 receptors, can, however, produce and release endocannabinoid ligands from their somas and dendrites, then facilitating

the retrograde signaling function of these molecules and CB_1 receptors in excitatory and inhibitory synapses (reviewed in [37] and see also below).

Despite that most of cannabinoid effects on DA transmission seem to be GABA- and/or glutamate-mediated, recent studies have provided an additional mechanism available for those eicosanoid-derived cannabinoids that have demonstrated some affinity for the $TRPV_1$ receptor (e.g., anandamide, AM404 or *N*-arachidonoyl-dopamine (NADA); reviewed in [38]). These receptors are molecular integrators of nociceptive stimuli, abundant on sensory neurons, but they have been found in dopaminergic neurons within the basal ganglia too [39], thus enabling a direct action of certain cannabinoids on DA function. In fact, there is recent evidence demonstrating that endovanilloid and DA signalling systems are closely linked in the regulation of various neurobiological processes including the control of movement [40,41]. The case of NADA deserves some comments since it is formed by a molecule of DA linked to arachidonic acid by an amide bond which conferes this molecule properties of endocannabinoid and endovanilloid ligand [38]. NADA

seems to be synthesized through the conjugation of an arachidonic acid molecule directly with DA [42], discarding previous hypothesis that suggested that it would be synthesized through the hydroxylation of *N*-arachidonoyl-tyrosine followed by decarboxylation by the same enzymes involved in DA synthesis. Its physiological significance is yet poorly understood, but recent evidence suggests that it can serve as an antioxidant and neuroprotective compound [43].

Finally, a recent study by Oz et al. [44] published during the preparation of this review has provided preliminary evidence that anandamide may inhibit the DA transporter function by a receptor-independent mechanism, an effect found in heterologous cells and synaptosomal preparations. The anandamide analog methanandamide mimicked this effect, but arachidonic acid was without effect [44]. In addition, inhibition of FAAH or COX-2 failed to alter the effect of anandamide, thus indicating that this effect is not related to the metabolism of this endocannabinoid [44]. Authors also found that the effect was not attenuated by pertussis toxin, then excluding the involvement of CB_1, CB_2, or GPR55 receptors, but this does not exclude that $TRPV_1$ receptors may be involved in line with the comments of the above paragraph.

Both aspects, that cannabinoids may alter DA transmission and DA-related behaviors via an indirect action on GABA and glutamate neurons, and that they can activate $TRPV_1$ receptors located onto dopaminergic neurons, will be key elements in this review. We will concentrate preferentially in reviewing the role(s) played by endocannabinoids on those adult brain functions, where DA is a key regulatory neurotransmitter, namely the control of motor function at the basal ganglia level, and the expression of some cognitive functions, including emotionality, motivation, and brain reward. Additional interactions between cannabinoids and DA have been also claimed for the hypothalamic regulation of pituitary hormone secretion [45], for the expression of key genes during brain development [16,17], for memory formation [46,47], sleep regulation [48], and retina function [49] in mammals, as well as some interactions in the brain of invertebrates [50]. However, they will not be addressed in the present review.

Interactions Between Cannabinoids and DA at the Basal Ganglia

Anatomical, physiological and therapeutic evidences indicate that DA is the key regulatory transmitter in the basal ganglia circuitry (reviewed in [51]). So, the activation of DA transmission in this circuitry is generally associated with an increase of movement, whereas the inhibition is followed by hypokinesia. In fact, the basal ganglia disorder with highest prevalence in the human population, PD, is consequence of a progressive degeneration of nigrostriatal dopaminergic neurons resulting in bradykinesia, rigidity, and tremor [52]. Cannabinoids are hypokinetic substances thus producing motor depression and even catalepsy (reviewed in [9]) and it has been largely speculated that this hypokinetic effect of cannabinoids might be produced by reducing dopaminergic activity. This assumption is correct but the role of CB_1 receptors, which are not located onto dopaminergic neurons [53], in this effect has not been completely elucidated. Meanwhile, the increasing importance of $TRPV_1$ receptors for the action of certain endocannabinoids [38], as well as the location of these receptors onto dopaminergic neurons [39], have opened interesting novel aspects to discuss about the role and therapeutic potential of the endocannabinoid signaling in the basal ganglia, from both basic and clinical perspectives, aspects that will be addressed below.

Cannabinoids, DA and the Basal Ganglia in Healthy Conditions

Behavioral Data

The abundant presence of CB_1 receptors and their endogenous ligands in brain regions related to the control of movement, such as the different basal ganglia (e.g., caudate-putamen, globus pallidus, and substantia nigra) and the cerebellum [2,21,34–36], suggests that the endocannabinoid system plays a prominent role in the control of movement (for review, see [8–10]). Thus, excluding very low doses which may produce stimulatory effects in some cases, it is generally well accepted that the different cannabinoid agonists originate a dose-dependent motor inhibition in both humans and laboratory animals, that may produce even catalepsia with the highest doses (reviewed in [8,9]). Similar results were obtained by administering inhibitors of the endocannabinoid inactivation, the so-called indirect cannabinoid agonists (reviewed in [9]). These hypokinetic effects were generally reversed by the administration of CB_1 receptor antagonists (e.g., rimonabant, despite some differences found depending on species used), while these antagonists produced by themselves a certain degree of hyperlocomotion due to their frequent properties as inverse agonists (reviewed in [8,9]). In concordance with these last data, mice lacking CB_1 receptors exhibited several motor anomalies (see [24] for review), thus supporting the idea that this receptor type is the key cannabinoid receptor involved in motor effects of cannabinoid compounds.

Neurochemical Data

The motor effects of cannabinoid agonists are likely originated because of the capability of these substances to influence the activity of several neurotransmitters, through the activation of CB_1 receptors located in specific neuronal subpopulations within the basal ganglia circuitry (see Figure 2). Striatal projection GABAergic neurons and subthalamonigral glutamatergic neurons, both containing CB_1 receptors [35,36,53,54] were identified first as the major substrates for the action of cannabinoids. More recent evidence suggest, however, that CB_1 receptors are also located in corticostriatal glutamatergic afferences [55,56] and in some subpopulations of striatal GABA interneurons [54,55]. It is important to remark that, in the basal ganglia, CB_1 receptors are always located in GABA or glutamate-containing neurons, which means that the first event associated with the activation of these receptors is an alteration in the activity of GABA and glutamate synapses. However, despite dopaminergic neurons within the basal ganglia do not contain CB_1 receptors (with the only exception of the developmental period [31,57]), they can be secondarily altered given that they are preferential targets for GABA and glutamate synapses. This is supported by numerous pharmacological studies that demonstrated how cannabinoid agonists strongly modified the motor effects of some DA-acting substances. For example, they potentiated reserpine-induced hypokinesia [58] and dopaminergic antagonist-induced catalepsy [59], while reduced quinpirole-induced hyperlocomotion [60] and amphetamine-induced hyperactivity [61], although these responses may change when low doses of cannabinoid agonists were used (reviewed in [9]). Neurochemical studies also sustain the idea that cannabinoids reduce the activity of nigrostriatal dopaminergic neurons (reviewed in [8,9,32]), although, as mentioned above, the lack of CB_1 receptors in these neurons would imply that the changes in the activity of dopaminergic neurons were originated by previous changes in GABAergic or glutamatergic influences arising the substantia nigra ([8,9] for review).

As mentioned above, further investigations have, however, provided new elements to re-evaluate the idea that the effects of endocannabinoids on DA transmission in the basal ganglia are exclusively indirect. For example, it has been demonstrated that anandamide and some analogs (e.g., AM404 and NADA), but not classic cannabinoids (e.g., Δ^9-THC), may behave as full agonists for the $TRPV_1$ receptors (reviewed in [38]). These receptors have been also identified in the basal ganglia (see Figure 2) in colocalization with tyrosine hydroxylase enzyme, which in these structures, serves as a specific marker for dopaminergic neurons [39]. $TRPV_1$ receptors

have been also identified in glutamatergic terminals in the substantia nigra pars compacta [62]. The activation of these receptors with capsaicin or with other potential vanilloid ligands produced hypokinesia in rats [63]. The same effect was seen with anandamide accompanied by a reduction in the activity of dopaminergic terminals in the striatum [64]. This effect of anandamide was reversed by capsazapine, thus indicating that it would be exerted by the activation of $TRPV_1$ receptors [64]. These neurochemical responses were furtherly confirmed *in vitro* using perfused striatal fragments, but they were not reproduced by classic cannabinoids, such as Δ^9-THC, that do not bind to vanilloid-like receptors [64], thus indicating that the $TRPV_1$ receptor rather than the CB_1 is the key target in these responses. By contrast, other authors [62,65] found that the activation of $TRPV_1$ receptors in the substantia nigra pars compacta stimulated DA release, although these effects might be mediated by $TRPV_1$ receptors located in glutamatergic neurons rather than by those located in dopaminergic terminals. Other support to the increasing relevance of $TRPV_1$ receptors in the basal ganglia comes from studies conducted in rat models of Huntington's disease, where several cannabinoid-based compounds, such as AM404, exhibited antihyperkinetic properties, being these effects depending on their capability to activate $TRPV_1$ receptors rather than CB_1 receptors [66,67]. Finally, making the issue even more complex, a recent study by Ferrara and coworkers [68] revealed that *N*-acyldopamines, such as NADA, are able to control the activity of dopaminergic terminals in the striatum via novel ion channels other than $TRPV_1$ receptors, an effect that was not observed with anandamide or capsaicin. Importantly, NADA was likely synthesized in the substantia nigra in conditions of hyperactivity [62].

Despite CB_1 receptors do not appear to be located in dopaminergic neurons, they colocalize with D_1 or D_2 receptors in striatal GABAergic projection neurons (striatonigral and striatopallidal pathways, respectively) [69,70], which facilitate postsynaptic interactions between endocannabinoids and DA at the level of G-protein/adenylyl cyclase signal transduction [71,72], even the formation of heteromers between CB_1 and D_2 receptors, and also adenosine A2a receptors (reviewed in [73]). These CB_1, D_2, and A2a receptor heteromers were found in the dendritic spines of GABAergic neurons projecting to the globus pallidus, but their functional properties and their role in striatal function are pending of further investigation (reviewed in [73]). This type of postsynaptic mechanisms facilitates the direct interaction between cannabinoids and DA allowing, in this case, a bidirectional regulation, endocannabinoids and DA and viceversa. It is in this context of bidirectional regulation where one may place, at the same time, data showing

that: (i) motor effects of CB_1 agonists are associated with an activation of DARPP-32 signaling, which has been linked to intracellular responses elicited by D_1 and D_2 receptors in the striatal projection neurons, whereas the genetic inactivation of DARPP-32 attenuated motor effects of cannabinoids [74]; and (ii) dopaminergic D_2 receptors control anandamide production in the striatum, thus indicating that the endocannabinoid system serves as an inhibitory feedback mechanism counteracting DA-induced facilitation of psychomotor activity [71]. In addition, D_2 receptors also control $G_{i/o}$ protein availability for CB_1 receptors [75] and facilitate endocannabinoid-mediated long-term synaptic depression of GABAergic neurons [76], an effect also seen in the ventral-tegmental area [77]. A similar interaction of endocannabinoids with D_1 receptors has been recently demonstrated [70]. All these observations are concordant with the old idea proposed by Mailleux and Vanderhaeghen [78] that endocannabinoid signaling in the basal ganglia is regulated by DA and viceversa, which might be relevant for a disease like PD, where CB_1 receptors and their ligands seem to be up-regulated in conditions of DA deficiency (see [79–81] and details later).

Cannabinoids, DA and the Basal Ganglia in Pathological Conditions

Assuming that the endocannabinoid signaling system modulates the activity of DA and other neurotransmitters at the basal ganglia by acting at CB_1 and/or $TRPV_1$ receptors, one may postulate that the pharmacological management of this system may serve to normalize DA transmission in conditions of DA deficiency, overactivity or dysregulation, and, subsequently, to alleviate DA-related motor symptoms in various basal ganglia disorders (for review, see [9,32,82]). To date, most studies that have addressed this issue are preclinical and have provided the first experimental evidences using animal models (see [10,82] for recent reviews). However, in some cases, a few number of clinical trials have been already conducted unfortunately with poorly effective results [83–88]. As this review is centered in cannabinoid-DA interactions, we will concentrate here in PD, the major basal ganglia disorder characterized by either dopaminergic degeneration or malfunctioning. However, we will briefly mention that cannabinoids have been also studied in other disorders related to the basal ganglia function, for example in Huntington's disease where direct or indirect (inhibitors of endocannabinoid inactivation) agonists of CB_1 receptors, especially if they also behave as $TRPV_1$ receptor agonists, have been proposed as having therapeutic value (reviewed in [89]). Cannabinoid agonists, presumably those activating CB_1 receptors, are also effective in Gilles de la Tourette's syndrome, where they reduced tics and improved behavioral problems in patients (reviewed in [90]). Similar studies have been conducted in relation with dystonia [84,91] and dyskinesia, particularly, the case of levodopa-induced dyskinesia [92].

As mentioned above, PD is the major basal ganglia disorder characterized by the progressive death of nigral dopaminergic neurons and DA denervation of the striatum. Both CB_1 receptor agonists and antagonists have been proposed of therapeutic value in this disorder, alone or as coadjuvants, and addressed to alleviate specific motor symptoms or to delay/arrest the progression of this disease (reviewed in [8,9,82,93]). CB_1 receptor agonists have been proposed, for example, for the reduction of tremor associated with the frequent overactivity of the subthalamic nucleus occurring in PD [94], although the few clinical trial conducted to explore this effect in patients did not generate positive results [88]. CB_1 receptor agonists were also investigated in relation with the dyskinetic states associated with long-term therapy of dopaminergic replacement with levodopa, showing positive effects [95]. However, this effect was not observed with the so-called indirect cannabinoid agonists, e.g., FAAH inhibitors, presumably because they are also able to activate $TRPV_1$ receptors [96]. Only using coadministration with a $TRPV_1$ receptor antagonist, FAAH inhibitors were capable to show antidyskinetic properties, thus indicating that CB_1 and $TRPV_1$ receptors have opposite effects in the control of levodopa-induced dyskinesia [96]. Again the clinical testing of this potential produced controversial results [83,97].

Positive effects were also found in the case of those plant-derived cannabinoid agonists having cannabinoid receptor-independent antioxidant properties, when they were examined for their capability to afford protection against dopaminergic degeneration in experimental models of parkinsonism [98,99], indicating that they may represent a promising therapy against disease progression in PD. These results provide a neurobiological support for those anecdotal data (e.g., surveys) that indicated that PD patients who self-medicated with cannabis obtained beneficial effects (see an example in [100]), despite controlled clinical studies did not reproduce these benefits [97]. However, both surveys on cannabis use for PD and clinical studies were always centered in specific symptoms rather than in the disease progression, therefore the issue deserves further clinical testing. Positive effects against disease progression have been recently obtained with CB_2 receptor agonists in MPTP-lesioned animals [101], whereas CB_1 receptor-deficient mice resulted to be more vulnerable to the lesion with 6-hydroxydopamine than wild-type animals [102], thus

indicating an additional contribution of CB_1 and CB_2 receptors in neuroprotective effects of cannabinoids in this disorder that would also deserve further clinical investigation.

By contrast, the blockade of CB_1 receptors (e.g., with rimonabant) was recently reported to reduce bradykinesia and other parkinsonian symptoms (e.g., levodopa-induced dyskinesia; see Figure 3 and below) [103–105], despite previous studies showing conflicting results [81,106] and the negative results found in the only clinical trial conducted with CB_1 receptor antagonists in parkinsonian patients [87]. It appears that the blockade of CB_1 receptors: (i) works more efficiently in certain circumstances, for example in very advanced phases of the disease characterized by extreme nigral damage [103], (ii) would be DA-independent [104] despite it enhanced the antiparkinsonian efficacy of levodopa [105,107], and/or (iii) needs the use of low doses of the antagonist [104,105] (see Figure 3), conditions that were not completely reproduced in the only clinical trial conducted so far with rimonabant which included a group of patients that were all well responders to levodopa [87]. Further studies have demonstrated that this effect of rimonabant would be exerted by enhancing glutamatergic transmission at the striatal level [108]. Therefore, if these preclinical data are finally confirmed with the appropriate clinical testing, it would be possible to have a novel antiparkinsonism agent in a stage of the disease when the classic dopaminergic therapy is generally failed or for the group of patients that have a poor levodopa response. CB_1 receptor antagonists have been also proposed for reducing and/or delaying levodopa-induced dyskinesia (reviewed in [92]), thus indicating the extreme complexity of this circuitry where both CB_1 agonists and antagonists may serve for the same type of treatment, a fact presumably related to the presence of these receptors in both excitatory and inhibitory synapses within the basal ganglia circuitry. On the other hand, it is important to remark that the usefulness of CB_1 receptor antagonists in this disease agrees with the type of pharmacological strategy expected once several studies have demonstrated an up-regulation of CB_1 receptors and other elements of this signaling system in PD [78–81]. For some authors, there is an unbalance between DA, which goes down, and endocannabinoids, which go up, at the basal ganglia once nigral damage is already evident (early stages in the development of parkinsonism, when nigral damage does not exist or is minimal, may be, however, associated with down-regulatory responses [109]), which supports the potential of CB_1 receptor antagonists in this disease. This type of response has been observed in rats treated acutely with reserpine [81] or chronically with dopaminergic antagonists [78], or after the damage of ni-

grostriatal neurons with 6-hydroxydopamine [78,79] or MPTP [80] in different laboratory animals. It was also found in patients [80,110]. In support of the idea of unbalance, the classic dopaminergic replacement therapy with levodopa reversed this endocannabinoid overactivity [80,111]. On the other hand, it is also important to consider the therapeutic benefits that can offer the antagonists of $TRPV_1$ receptors for the treatment of motor anomalies in PD, given their recently demonstrated role in regulating DA release from nigral neurons [64]. For example, they have been recently found to be necessary for unmasking the antidyskinetic effecs of FAAH inhibitors or other cannabinoid agonists capable to directly or indirectly activate $TRPV_1$ receptors [96]. However, given that they are located in the neuronal subpopulation that degenerates in this disease [97], it is possible that this target may loss interest as soon as the disease progresses, something important in a disease whose first motor symptoms appear when an important loss of dopaminergic neurons has already occurred.

Interactions Between Cannabinoids and DA at the Corticolimbic Structures

Mesocorticolimbic dopaminergic neurons play a regulatory function in the control of cognitive processes, motivated behavior, the central stress response, and the pleasure produced by natural (e.g., sex, food) or other types (e.g., drugs of abuse) of reinforcers, or by compulsive activities, such as gambling, overeating and sex dependence (for review, see [112,113]). In fact, the neurotransmitter more studied as a potential target for the pharmacological effects of habit-forming drugs is DA (for review, see [114]). This also includes the case of cannabis derivatives (for review, see [115,116]), which acting through cannabinoid receptors, likely the CB_1 receptor type, are able to alter mesocorticolimbic DA transmission. Therefore, a first level of interaction between cannabinoids and DA in corticolimbic structures can be found in processes like brain reward and drug abstinence/dysphoric responses [115–119]. However, the interactions of cannabinoids and DA at this level are probably largest and involve more cortical and subcortical structures and more types of processes. From a pharmacological point of view, cannabinoid agonists (depending on doses and duration of treatment) produce euphoria, stimulate brain reward, are anxiolytic, and decrease motivation and arousal while increasing emotionality, effects that were observed in humans and laboratory animals (reviewed in [120,121]). These effects were paralleled by alterations in mesocorticolimbic dopaminergic neurons, but it is generally accepted
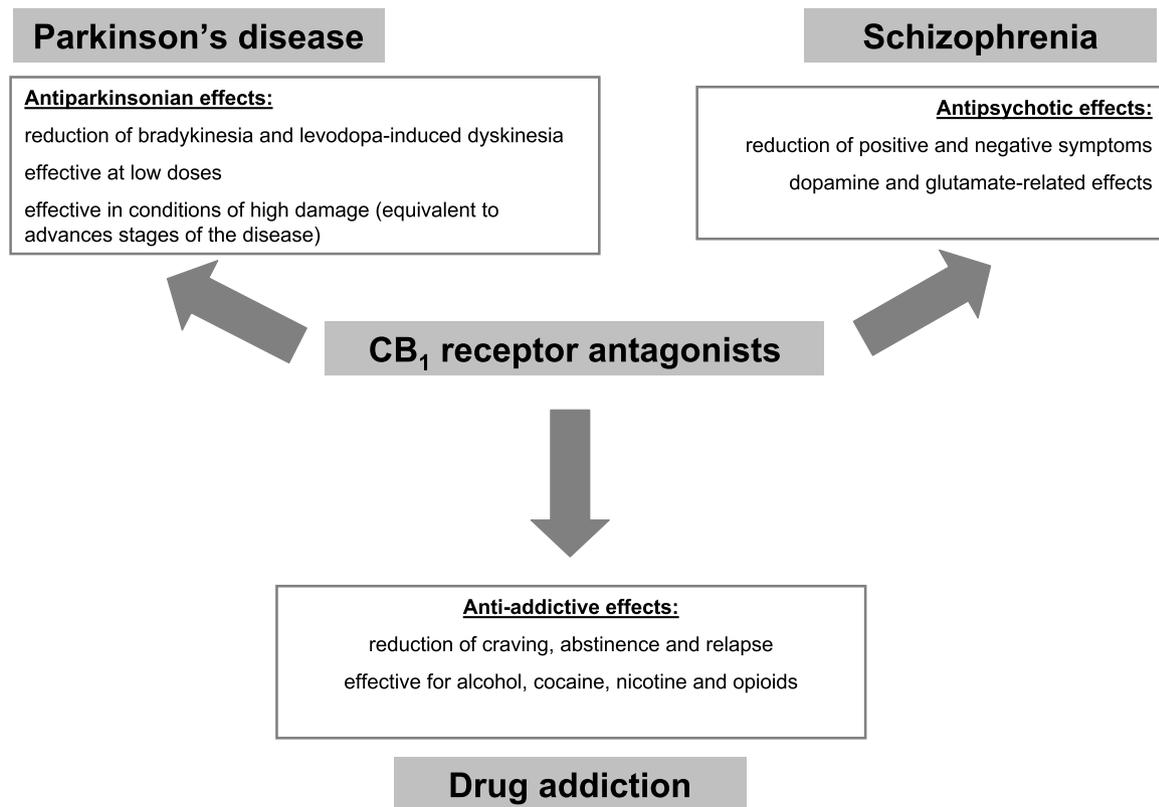
Parkinson's disease

Antiparkinsonian effects:

reduction of bradykinesia and levodopa-induced dyskinesia

effective at low doses

effective in conditions of high damage (equivalent to advances stages of the disease)

Schizophrenia

Antipsychotic effects:

reduction of positive and negative symptoms

dopamine and glutamate-related effects

CB$_1$ receptor antagonists

Anti-addictive effects:

reduction of craving, abstinence and relapse

effective for alcohol, cocaine, nicotine and opioids

Drug addiction

Figure 3 Summary of potential therapeutic applications proposed for CB$_1$ receptor antagonists in pathologies related to dopamine transmission in the CNS.

that DA transmission is not the first target for the action of cannabinoid agonists also in these structures, so that the effects would be most likely indirect [120,121], as in the case of the basal ganglia. Several authors proposed glutamatergic and/or GABAergic inputs to the nucleus accumbens/prefrontal cortex and ventral-tegmental area as the primary targets for the cannabinoid action in these processes and also as the responsible of DA changes [122–124]. These glutamatergic and GABAergic subpopulations would contain CB$_1$ receptors regulating presynaptic events and would be ultimately in contact with dopaminergic neurons (for review, see [121]). This includes, for example, the recent demonstration of CB$_1$ receptors in excitatory projections coming from subcortical structures and reaching the bed nucleus of the stria terminalis which, in turn, projects to the ventral-tegmental area [125]. Finally, there is some evidence of colocalization of CB$_1$ receptors and tyrosine hydroxylase in the ventral-tegmental area [30], where cell bodies of mesocorticolimbic dopaminergic neurons are located, which opens the possibility of a direct action of cannabinoids on the major substrate for brain reward, motivation/emotionality and other limbic processes.

Cannabinoids, DA, and Corticolimbic Structures in Healthy Conditions

Behavioral Data

As mentioned above, CB$_1$ receptors are located in glutamatergic and/or GABAergic synapses within cortical and subcortical structures and they represent the major substrate for cognitive effects and reinforcing properties of cannabinoids administered to laboratory animals or consumed by human subjects. These pharmacological effects coincide with two key functions described for the cannabinoid signaling system in corticolimbic structures. In relation with the cognitive effects of cannabinoids, it is well known that CB$_1$ receptors are moderately abundant in different cortical structures, in particular they are located in superficial and deep layers, presumably onto GABAergic interneurons [3,34–36] and the same happens with their endogenous ligands [21]. These anatomical data suggest a role for the cannabinoid signaling system in the control of sleep-waking cycle, performance of complex cognitive tasks, working memory, temporal organization of behavior, adaptation of behavioral strategies, sensory perception, and other

cognitive functions, whose control resides mainly in the different cortical structures (for review, see [126]). This provides an explanation for major subjective effects and cognitive impairments experienced by cannabis consumers, including: (i) the case of naïve consumers in which cannabinoids reversibly impair cognitive functions, a phenomenon also demonstrated in laboratory animals (reviewed in [127]), and (ii) the particular case of long-term marijuana abuse where severe irreversible deficits in cognitive function and precipitation of psychiatric disorders, such as psychosis, anxiety or depression, have been postulated, in particular when the abuse of marijuana starts at very early ages during the adolescence (reviewed in [127–129] and see below). Anyway, the evidence surrounding the cognitive effects of cannabis is always a matter of continuous debate, with studies stating the critical role played by cannabinoids in the development of psychiatric disorders for the general population and others that circumscribe this possibility only in vulnerable individuals (reviewed in [127–129] and see below).

The cannabinoid signaling system also plays a role in brain reward processes activated by different types of reinforcers, particularly, the addictive drugs, including the case of cannabis consumption itself [130] but also of other different drugs of abuse [131,132]. This assumption is supported by several evidences. First, several neuroanatomical studies have demonstrated that elements of the cannabinoid signaling system, particularly the CB_1 receptors, are abundant in the different brain structures that form the brain reward circuitry [21,30,34–36]. Second, several biochemical studies have demonstrated that these elements, again in particular the CB_1 receptor, experience important changes in conditions of acute exposure, chronic consumption, dependence, abstinence, or relapse for most frequently consumed habit-forming drugs, including the case of opioids [133–135], cocaine [133,136], nicotine [136] or alcohol [133,136–139]. The importance of a role of the cannabinoid system in addictive processes is that it opens the possibility that the pharmacological management of the cannabinoid signaling may serve to improve behavioral and/or neurochemical anomalies occurring during addictive states (see next section), and for this potential, the interactions between cannabinoids and DA seem to be critical.

Neurochemical Data

Several cannabinoid agonists, mainly Δ^9-THC, the major psychoactive ingredient of cannabis, have been reported to increase mesolimbic dopaminergic activity (reviewed in [115]), as demonstrated in numerous studies conducted with laboratory animals in which the treat-

ment with different cannabinoid agonists elevated D_1 receptor density, DA release, and DA metabolism in various limbic structures, as well as it enhanced the firing rate of mesolimbic dopaminergic neurons in the A10 region (reviewed in [129,140]). The involvement of the CB_1 receptor seems critical for these effects and explains why knockout mice for the CB_1 receptor exhibited reduced voluntary alcohol consumption [141], morphine self-administration [142,143], cocaine-enhanced locomotion [144], and absence of rewarding effects of nicotine evaluated in the conditioned place preference test [145], although they did not show similar responses for cocaine or nicotine reinforcement in the self-administration paradigm [143]. Again, DA transmission seems to be critical here because the effects of alcohol, morphine, cocaine, or nicotine on DA release in the nucleus accumbens were completely absent in CB_1 receptor knockout mice [141,142,144,146]. In the same direction, an increased endocannabinoid tone has been demonstrated to facilitate the effects of most commonly abused drugs on DA transmission, since DA release was uniformly inhibited by the blockade of CB_1 receptors [147]. However, adding complexity to this issue, a recent study by Melis et al. [148] demonstrated that FAAH inhibitors were also effective in the reduction of nicotine-induced activation of dopaminergic neurons, which contradicts the idea that the endocannabinoid system, and particularly the CB_1 receptor, exerts a stimulatory effect on nicotine reinforcing properties. In this case, FAAH inhibitors would act by enhancing the action of certain signaling *N*-acylethanolamines (e.g., oleoylethanolamide and palmitoylethanolamide), which are devoid of CB_1 activity, but are capable to act at the PPAR-α nuclear receptors, opening a new avenue in the understanding and perhaps in the treatment of drug addiction.

In parallel to their effects on mesolimbic projections neurons, cannabinoid agonists also augmented the activity of those dopaminergic neurons that coming from the ventral-tegmental area project specifically to the prefrontal cortex (reviewed in [149]). The prefrontal cortex is involved in many cognitive functions, including working memory, temporal organization of behavior, and adaptation of behavioral strategies, that, as mentioned above, are affected by cannabinoids [149]. This region contains a moderate density of CB_1 receptors [34,35]. The acute administration of cannabinoids increased DA release in rat prefrontal cortex, measured by *in vivo* microdialysis, but this effect is produced presumably by a previous increase of glutamate release and/or a decrease of GABA, given that CB_1 receptors are not located in dopaminergic neurons [150]. By contrast, the repeated administration of cannabinoid agonists decreased DA turnover in the prefrontal cortex but not in

the nucleus accumbens and the striatum [151,152], an effect that persisted even after a drug-free period of 2 weeks [152].

In general, there are no discrepancies concerning that the activation of CB_1 receptors is involved in most of effects produced by cannabinoids on mesocorticolimbic activity. However, as mentioned above, these effects have been considered, so far, as exerted indirectly [132]. Possibly, they would be caused through modifying GABAergic influences to the ventral-tegmental area and/or the nucleus accumbens, given that CB_1 receptors have been found in these GABA neurons rather than in mesocorticolimbic ones [117,153,154]. These GABA neurons tonically inhibit DA-containing neurons and an inhibition of GABA release by cannabinoid agonists, via presynaptic CB_1 receptors, would be expected to increase the activity of dopaminergic neurons [123,124,154–158]. Alternatively, CB_1 receptors may be located in the excitatory glutamatergic inputs to the GABA-containing neurons that project from the nucleus accumbens to the ventral-tegmental area, as reported by Melis and coworkers [159]. In this case, the activation of CB_1 receptors would result in a decrease of glutamate release followed by reduction in GABA activity and, again, in an increase in the firing of dopaminergic neurons [124,160]. An important aspect to remark is that, in both cases– CB_1 receptors located in GABA- or glutamate-containing neurons– the activation of these receptors would depend on the release of endocannabinoids by dopaminergic neurons, a fact associated with the increase in the activity of these neurons provoked by abused drugs [121]. According to these authors [121], the release of endocannabinoids and the subsequent activation of presynaptic CB_1 receptors by these signaling lipids may represent a common phenomenon associated with the action of a wide variety of habit-forming drugs. Finally, it is also important to consider the study published by Wenger and coworkers [30] who, as mentioned above, demonstrated for the first time, using double immunohystochemistry, that CB_1 receptors colocalize with tyrosine hydroxylase in the nucleus accumbens. This opens the possibility of a direct action of cannabinoids on the major neurochemical substrate of brain reward, dysphoria-mediated drug craving and drug relapse, although not all researchers agree with this possibility (reviewed in [121,140]).

Cannabinoids, DA and Corticolimbic Structures in Pathological Conditions

As in the case of the basal ganglia, the capability of the cannabinoid system to influence DA transmission in corticolimbic structures supports that the pharmacological management of this system might have therapeutic value in those diseases involving anomalies of mesocorticolimbic DA transmission, among them, addictive states, and schizophrenia and other psychosis. The working hypothesis is that normalizing DA transmission, with either cannabinoid agonists or antagonists depending on the type of dysfunction, would result in reducing addictive processes or producing antypsychotic effects.

As mentioned earlier, the endocannabinoid transmission has been related to many signs of drug addiction. For example, it can be related to cases of individual vulnerability for drug abuse, a fact supported by recent data showing a higher occurrence of this disorder in individuals bearing specific genetic variants of the CB_1 receptor or the FAAH enzyme, particularly for the case of alcohol and opioids [161–163]. However, most of the evidence supporting a relation of the cannabinoid system with drug addiction involve processes of craving, degree of dependence and intensity of abstinence, or risk to relapse for different types of drugs [119,164–168]. The importance of this fact is that it opens the possibility of using cannabinoid-related substances in the treatment of different aspects of drug addiction (for review, see [131,132,137]). These effects would be exerted, among others, by normalizing DA transmission, in particular in those addictive responses more directly related to changes in DA activity such as reinforcement/relapse [118] or withdrawal/dysphoria-mediated drug craving [117]. Most of pharmacological studies have concentrated in the potential of CB_1 receptor antagonists and the most-studied compound has been rimonabant [169] (see Figure 3). For example, the blockade of CB_1 receptors with this antagonist impaired the perception of reinforcing potential of different habit-forming drugs [170], indicating that positive incentive and/or motivational processes (and relapsing properties too) could be under a permissive control of CB_1 receptor-related mechanisms. This has been described for many drugs including the case of alcohol [141,167,171,172], morphine [164,165,173,174] and nicotine [146,175,176], and specifically for relapsing properties in the case of cocaine [166,177]. This concept has been recently extended to physical dependence/abstinence for most of these drugs (reviewed in [169]). This pharmacological potential shown by rimonabant (and other CB_1 receptor antagonists) in preclinical studies led to an exhaustive clinical testing, particularly for tobacco addiction (STRATUS clinical trial, see [169,178]) and, more recently, for alcohol dependence (ACTOL clinical trial, see [179]), although the results were not positive enough. Major problems with rimonabant and other similar antagonists are that they behave also as inverse agonists, and, given the ubiquitous distribution of CB_1 receptors within the CNS, the use of this type of compounds in

the clinic is frequently associated with the occurrence of psychiatric side effects. Therefore, the matter is pending of the development of neutral CB$_1$ receptor antagonists [169].

Cannabinoids–DA interactions are also important in another disorder related to corticolimbic structures such as schizophrenia. There are multiple lines of research relating cannabinoids to DA transmission in schizophrenia. For example, the brain structures involved in the pathogenesis of schizophrenia (limbic areas, prefrontal cortex), structures that are densely innervated by dopaminergic terminals, contain also a moderate but significant density of CB$_1$ receptors [34–36]. They also contain relevant amounts of endocannabinoids [21]. This may explain why cannabis consumption has been associated with the induction or enhancement of psychosis in various cohort studies, and, then, proposed as a potential risk factor for the development of this disorder, in particular in the case of heavy abusers (e.g., frequent consumers or consumers of high Δ^9-THC cannabis), early consumers (e.g., adolescence is a particularly vulnerable period) and/or predisposed individuals (e.g., subjects with family history or prodromal symptoms, or bearing the Val allele of COMT) (reviewed in [127–129]). Cannabis consumption has been also associated with more frequent and earlier relapses (reviewed in [180]). However, as mentioned above, the association between cannabis and psychosis is not completely understood (see [127–129,181,182] for review). It is also important to remark, given the objective of this review, that, for some authors, cannabinoid agonists, independently of their capability to induce or aggravate psychotic episodes, seem to be capable to interfere with classic antipsychotics, thus reducing their capability to block D$_2$ receptors and/or exacerbating their extrapyramidal symptoms [183,184]. However, for some authors, this can be a sort of self-medication to diminish neuroleptic-side effects [129,181]. That cannabis/cannabinoids may cause or exacerbate psychoses may be related to the facilitatory effects of cannabinoids on DA transmission at the nucleus accumbens [181], given that overactivity of DA transmission in this structure is a key pathological event in schizophrenia. The inhibitory effect of cannabinoids on GABA and glutamate transmission may also collaborate in these responses [129].

Another relevant observation concerning the possible relation of cannabis/cannabinoids with the pathogenesis of schizophrenia was obtained in genetic studies, which revealed the association of a polymorphism of the CB$_1$ receptor gene with increased susceptibility to develop schizophrenia [181,185–187], although there are studies showing no association (reviewed in [181]). For some authors, this is an important support to the idea that the cannabinoid signaling system plays a role in the pathogenesis of schizophrenia, at least in a subgroup of schizophrenic individuals [181]. This is the so-called cannabinoid hypothesis of schizophrenia, which would consist of an impairment in the activity of this signaling system (higher CB$_1$ receptor density or endocannabinoid levels, responses that have been found in schizophrenic patients; see below) in cortical and subcortical (limbic) structures. These changes would be associated with hyperactivity of dopaminergic neurons (positive symptoms) and hypoactivity of glutamate neurons (negative symptoms) (for review, see [32]).

On the other hand, despite the evidence indicating that cannabis consumption or cannabinoid signaling dysregulation may be adverse elements collaborating in the development of schizophrenia or reducing the efficacy of classic antipsychotic therapies, recent evidences have also indicated that certain cannabinoid-related compounds may serve as novel therapeutic agents in schizophrenia. This includes first the possibility that psychotic patients use cannabis to overcome the unpleasant feelings produced by classic antipsychotic therapy (reviewed in [181]). For example, Voruganti et al. [188], using *in vivo* SPECT analysis, demonstrated that cannabis smoking in schizophrenic patients produced an immediate calming effect, although followed by a worsening of psychotic symptoms [188]. The calming effect correlated with a reduction in striatal D$_2$ receptor binding that the authors interpreted as suggestive of increased DA activity [188]. Similar results were furtherly obtained by Bossong et al. [189], but not by Stokes et al. [190]. More recent evidences have indicated that the management of the cannabinoid signaling may even provide antipsychotic effects, with two compounds, cannabidiol and rimonabant, being the most studied. The antipsychotic potential of cannabidiol has been recently demonstrated even in the clinical area [191], although it is possible that its effects are not mediated by the activation of CB$_1$ or CB$_2$ receptors for which this phytocannabinoid has poor affinity, but through its capability to modulate TRPV$_1$ receptors or to interfere with endocannabinoid inactivation. As regards to rimonabant or other similar CB$_1$ receptor antagonists (see Figure 3), their antipsychotic potential was recently examined in preclinical models [192,193] at the light of the so-called cannabinoid hypothesis of schizophrenia mentioned above, according to which the cannabinoid signaling system would be elevated in schizophrenic patients. In this respect, schizophrenic patients have elevated anandamide levels in CSF [194,195] and blood [196]. Using analyses in postmortem human cortical and subcortical structures or neuroimaging procedures, several authors demonstrated that the density of CB$_1$ receptors is also elevated [197–200], although this

was not found in other study [201] and, even, a last study described a decrease in CB$_1$ receptor binding [202]. The preclinical studies revealed that the antipsychotic potential of rimonabant was related to alterations in DA [203] and glutamate [193] transmissions in cortical structures. However, rimonabant did not differ from placebo in reducing positive and negative symptoms in a placebo-controlled clinical trial conducted with patients having schizophrenia and schizoaffective disorder [204].

Concluding Remarks

Along this review, we have reviewed the classic knowledge and the recent advances on cannabinoid-DA interactions paying emphasis in two processes where DA has been proposed as a key neurotransmitter, such as the basal ganglia function and the corticolimbic processes. We have explored the mechanisms underlying these interactions, which represent the way for plant-derived cannabinoids to interfere with these processes. In most of the cases, we have concluded that dopaminergic neurons do not contain CB$_1$ receptors, with some exceptions, but these receptors are located on neurons present in regions innervated by dopaminergic neurons, which allows relevant bidirectional interactions. Finally, we have reviewed those diseases characterized by either deficiency, dysregulation or overactivity of DA transmission and where cannabinoids might be of therapeutic potential possibly through actions that facilitate, among others, a normalization of DA transmission. These diseases included basal ganglia disorders (e.g., Parkinson's disease), drug addiction, and schizophrenia and related-psychoses.

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Conflict of Interest

All authors declare that they have no conflicts of interest.

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