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The endocannabinoid system in the basal ganglia and in the mesolimbic reward system: implications for neurological and psychiatric disorders

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Abstract

To date, *N*-arachidonoylethanolamine (anandamide) and 2-arachidonoylglycerol are the best studied endocannabinoids and are thought to act as retrograde messengers in the central nervous system (CNS). By activating presynaptic cannabinoid CB_1 receptors, they can reduce glutamate release in dorsal and ventral striatum (nucleus accumbens) and alter synaptic plasticity, thereby modulating neurotransmission in the basal ganglia and in the mesolimbic reward system. In this review, we will focus on the role of the endocannabinoid system within these neuronal pathways and describe its effect on dopaminergic transmission and vice versa. The endocannabinoid system is unlikely to directly affect dopamine release, but can modify dopamine transmission trough trans-synaptic mechanisms, involving γ-aminobutyric acid (GABA)-ergic and glutamatergic synapses, as well as by converging signal transduction cascades of the cannabinoid and dopamine receptors. The dopamine and endocannabinoid systems exert a mutual control on each other. Cannabinergic signalling may lead to release of dopamine, which can act via dopamine D_1-like receptors as a negative feedback mechanism to counteract the effects of activation of the cannabinoid CB_1 receptor. On the other hand, dopaminergic signalling via dopamine D_2-like receptors may lead to up-regulation of cannabinergic signalling, which is likely to represent a negative feedback on dopaminergic signalling. The consequences of these interactions become evident in pathological conditions in which one of the two systems is likely to be malfunctioning. We will discuss neurological and psychiatric disorders such as Parkinson's and Huntington's disease, drug addiction and schizophrenia. Furthermore, the possible role of the endocannabinoid system in disorders not necessarily depending on the dopaminergic system, such as eating disorders and anxiety, will be described.

Keywords: Anandamide; 2-Arachidonoylglycerol; Cannabinoid; Dopamine; Movement disorder; Reward

1. Introduction

Marijuana is one of the most widely used illegal drugs throughout the world. It can induce strong behavioral effects such as depression of locomotor activity, and may have rewarding properties (Adams and Martin, 1996). The cannabinoid CB_1 receptor is thought to be responsible for the majority of the effects in the central nervous system (CNS) elicited by Δ^9-tetrahydrocannabinol (THC), the main psychoactive cannabinoid in marijuana (Gaoni and Mechoulam, 1964). The cannabinoid CB_1 receptor is one of the most abundant G-protein-coupled receptors in the CNS (Matsuda et al., 1990). Activation of presynaptic cannabinoid CB_1 receptors by its endogenous ligands, the endocannabinoids: *N*-arachidonoylethanolamine (anandamide) (Devane et al., 1992; Di Marzo et al., 1994) and 2-arachidonoylglycerol (Mechoulam et al., 1995; Sugiura et al., 1995), results in inhibition of both excitatory and inhibitory neurotransmitter release (Schlicker and Kathmann, 2001). The life span of endocannabinoids in the extracellular space is limited by a rapid elimination process consisting of selective uptake into the cell and subsequent degradation by fatty acid amide hydrolase or monoacylglycerol (MAG) lipase (Beltramo et al., 1997; Cravatt et al., 1996; Dinh et al., 2002). The cannabinoid receptors, endocannabinoids and the proteins for their biosynthesis and degradation constitute the endocannabinoid system (Di Marzo et al., 1998). This system is implicated in the regulation of various processes, such as learning, food uptake, pain transduction, reinforcement and motor coordination (Ameri, 1999). Its involvement in path-

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ophysiological conditions is beginning to be unravelled and might include disorders such as Parkinson's disease, Huntington's disease, drug addiction, schizophrenia, anorexia and obesity (Van der Stelt et al., 2002; Glass, 2001; Maldonado, 2002).

The endocannabinoid system has close connections with the dopaminergic system, which might explain several of its (patho)physiological aspects. The dopaminergic system is thought to play an important role in several psychiatric and neurological disorders. Dopamine is the predominant catechol neurotransmitter in the CNS and regulates a variety of functions such as locomotor activity, emotion and reward (Missale et al., 1998). Its involvement in movement control is emphasized in Parkinson's disease, where the loss of nigrostriatal dopaminergic neurons leads to severe motor deficits (Blandini et al., 2000). Mesolimbic dopaminergic neurons are also thought to have an important function in the acquisition of behavior that is reinforced by natural rewarding stimuli and substances of abuse (Spanagel and Weiss, 1999; Wise, 2002). In this paper, we will review the involvement of the endocannabinoid system with several neurological and psychiatric disorders, thereby paying special attention to its interactions with dopaminergic systems.

2. The endocannabinoid system

To date, the family of endocannabinoids is expanding. There are at least five different arachidonoyl derivatives, which can activate the cannabinoid receptor (Fig. 1). Anandamide and 2-arachidonoylglycerol are the two best studied members. 2-Arachidonoylglyceryl ether (noladin ether) (Hanus et al., 2001), *O*-arachidonoylethanolamine (virodhamine) (Porter et al., 2002) and *N*-arachidonoyldopamine (Huang et al., 2002) have only been recently identified as endogenous ligands for the cannabinoid receptors and their classification as true endocannabinoids awaits further biochemical and pharmacological characterization. It is widely recognized that endocannabinoids are not stored in vescicles like other mediators but, by analogy with other eicosanoids, are produced "on demand" (Di Marzo et al., 1994). This is the result of a biosynthetic mechanism relying on the existence of phospholipid precursors for these compounds, and of Ca^{2+}-sensitive and, possibly, G-protein-activated (phospho)lipases for the conversion of these precursors into their endocannabinoid products. The biosynthesis of endocannabinoids is immediately followed by their release. Although the biosynthetic routes underlying the formation of endocannabinoids have been extensively studied, the responsible proteins have not been cloned or purified to homogeneity and their regulation is far from clear (see Di Marzo et al., 1998 for a review). It is unknown which type of neurons express the biosynthetic enzymes, and, therefore, a classification of 'cannabinergic' neurons is not yet possible. Endocannabinoids can be inactivated via a two-step mechanism. Firstly, they are proposed to be translocated into the cell via selective transporters. Secondly, once inside the cell, they can be subjected to several different metabolic pathways such as hydrolysis by fatty acid amide hydrolase and monoacylglycerol lipase or oxygenation by lipoxygenases, cyclooxygenases and cytochrome P_{450}'s (for a review, see Van der Stelt and Di Marzo, in press).

To date, two G-protein-coupled receptors have been cloned, which can be activated by endocannabinoids (Matsuda et al., 1990; Munro et al., 1993). They are classified as cannabinoid CB_1 and CB_2 receptor. In the brain, the cannabinoid CB_1 receptor is found in areas controlling motor, cognitive, emotional and sensory functions, i.e., the hippocampus, basal ganglia, cerebellum, cortex and olfactory bulb (Herkenham et al., 1990; Tsou et al., 1998). It is thought that within the basal ganglia, cannabinoid CB_1 receptors are synthesized in the striatum and mostly transported to its

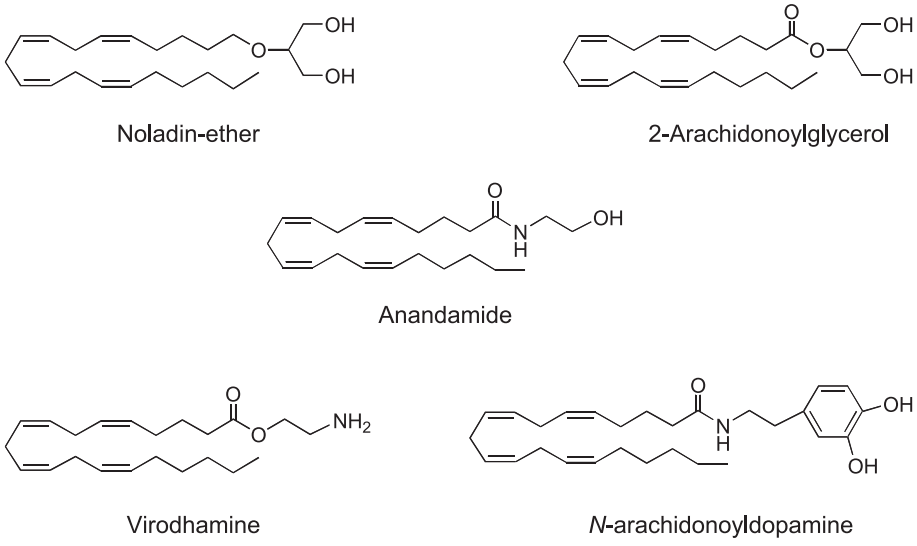

Fig. 1. Structures of endocannabinoids.

target areas, i.e., globus pallidus and substantia nigra (Herkenham et al., 1991a). Small nuclei with high density of cannabinoid CB_1 receptors are also found in other areas, for example, those controlling pain, body temperature, sleep–wake cycles and hormone function, such as the brainstem, the hypothalamus and the pituitary gland (Herkenham et al., 1991b). The cannabinoid CB_2 receptor seems to be confined to cells of the immune system. The existence of non-cannabinoid CB_1, non-CB_2 G-protein-coupled receptors for endocannabinoids, and anandamide in particular, has been suggested based on pharmacological and biochemical data (Breivogel et al., 2001; Di Marzo et al., 2000c; Sagan et al., 1999; Venance et al., 1995). It should be noted that also several non-G-protein-coupled molecular targets for anandamide have been proposed (Howlett and Mukhopadhyay, 2000). They are all membrane cation channels and are usually modulated specifically by anandamide at low or sub-micromolar concentrations. In particular, TASK-1 (TWIK-related acid-sensitive K^+ channel) K^+ channels (Maingret et al., 2001) and T-type Ca^{2+} channels (Chemin et al., 2001) are blocked by anandamide, whereas vanilloid type 1 (VR1) receptors, the sites of action of the pungent component of 'hot' red peppers, capsaicin, are activated by this endocannabinoid (Smart and Jerman, 2000; Zygmunt et al., 1999), as well as by *N*-arachidonoyl-dopamine (Huang et al., 2002).

Both cannabinoid CB_1 and CB_2 receptors inhibit cAMP formation via G_i-proteins and activate mitogen-activated-protein kinases (Pertwee, 1997). In addition, CB_1 receptors activate ion channels such as A-type and inwardly rectifying potassium channels, and inhibit voltage sensitive N-type and P/Q-type Ca^{2+} channels (Deadwyler et al., 1993, 1995; Di Marzo et al., 1998). An important functional consequence of the activation of presynaptic cannabinoid CB_1 receptors is the inhibition of neurotransmitter release (reviewed by Schlicker and Kathmann, 2001). In fact, endocannabinoids are proposed to act as retrograde messengers, thereby playing a crucial role in synaptic plasticity (Kreitzer and Regehr, 2001; Ohno-Shosaku et al., 2001; Wilson and Nicoll, 2001). Depolarisation of the post-synaptic membrane of neurons is thought to release endocannabinoids, which then diffuse to the presynaptic cannabinoid CB_1 receptors and inhibit their glutamatergic or γ-aminobutyric acid (GABA)-ergic input. Activation of presynaptic cannabinoid CB_1 receptors has been shown to be involved in long-term depression of neurons in the dorsal striatum and in the nucleus accumbens (Gerdeman et al., 2002; Robbe et al., 2002). Depending on the neuronal circuit, endocannabinoids may thus activate or inhibit neurotransmission.

3. Interactions between dopaminergic transmission and the endocannabinoid system in the nigrostriatal pathway

The basal ganglia consist of four nuclei: dorsal striatum (caudate and putamen), globus pallidus, substantia nigra and subthalamic nucleus, which play a major role in normal voluntary movement (Fig. 2). They do not have direct input or output connections with the spinal cord, but they receive their primary input from the cerebral cortex and send their output via the thalamus back to the cortex. The primary input center is the dorsal striatum, which controls planning and execution of motor behavior. In these region, glutamatergic projections from the sensorimotor, limbic structures (including amygdala), thalamus and dopaminergic input from the substantia nigra converge and are integrated by medium spiny neurons and redistributed to other nuclei of the basal ganglia. The medium spiny neurons send GABAergic projections to the globus pallidus and substantia nigra, which are the two major output centers. The globus pallidus and the substantia nigra tonically inhibit their target nuclei in the thalamus (Blandini et al., 2000).

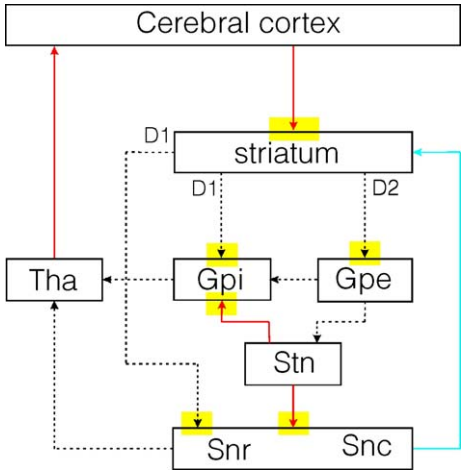

Fig. 2. Simplified scheme of the functional organization of the basal ganglia. The basal ganglia consist of four nuclei: dorsal striatum (caudate + putamen), globus pallidus (which is separated in an internal layer (Gpi) and an external layer (Gpe)), subthalamic nucleus (Stn) and substiantia nigra (which is separated into two parts, i.e., substantia nigra pars reticulata (Snr) and substantia nigra pars compacta (Snc)). The striatum is the primary input nuclei and contains GABAergic medium spiny neurons that receive glutamatergic innervation (red arrows) from the cerebral cortex and dopaminergic projections from the substantia nigra pars compacta (blue arrow). In the direct pathway, the medium spiny neurons of the striatum send their GABAergic projections (black arrows) to the substantia nigra pars reticulata and the globus pallidus interna (also called entopenducular nucleus), which are the two main output nuclei. The thalamus (Tha) is under inhibitory control of these output nuclei. In the indirect pathway, the medium spiny neurons send their GABAergic projections first to the external layer of the globus pallidus, which, in turn, inhibits the subthalamic nucleus with GABAergic efferents. The subthalamic nucleus activates the output nuclei with glutamatergic projections. It should be noted that the neurons in the substantia nigra pars compacta extend their dendrites into the substantia nigra pars reticulata, therefore, they are able to synapse with efferents from the striatum and subthalamic nucleus. The cannabinoid CB_1 receptor (yellow squares) is expressed in the different nuclei of the basal ganglia, thereby modulating neurotransmission in the direct (dopamine D_1 receptor expressing medium spiny neurons) and indirect (dopamine D_2 receptor expressing medium spiny neurons) pathways. See text for a more detailed description of the role of the endocannabinoid system in the basal ganglia. Figure is adapted from Blandini et al. (2000).

In a simplified model of the functional organization of the basal ganglia (Fig. 2), the inhibitory output is thought to be modulated by two parallel GABAergic pathways that run from the striatum to the output nuclei, i.e., the indirect and direct pathway (for a comprehensive review, see Blandini et al., 2000). The indirect pathway goes first to the external layer of the globus pallidus and from there to the subthalamic nucleus with GABAergic projections, and from the subthalamic nucleus to the internal globus pallidus (also termed entopeduncular nucleus in rodents) and substantia nigra with glutamatergic efferents. The direct pathway has two branches, one going first to the internal layer of the globus pallidus and then to the thalamus, and the other to the substantia nigra pars reticulata and then to the thalamus. The medium spiny striatal neurons that directly project to the output nuclei express dopamine D_1-like receptors, whereas those neurons that project to nuclei of the indirect pathway express the dopamine D_2-like receptor. Activation of the direct pathway dysinhibits the thalamus (facilitation of movement), whereas activation of the indirect pathway further inhibits the output centers (inhibition of movement). Activation of both dopamine D_1-like and D_2-like receptors in the two pathways reduces the inhibition of the output nuclei and enhances movement, because dopamine D_2-like receptors, which are negatively coupled to adenylate cyclase, inhibit the activity of the indirect pathway, while the direct pathway is activated by dopamine D_1-like receptors, which stimulate adenylate cyclase (Blandini et al., 2000).

Activation of the cannabinoid CB_1 receptor with low doses of agonists characteristically induce hyperactivity, while high doses lead to a depression of motor activity and catalepsy (Chaperon and Thiebot, 1999; Sanudo-Pena et al., 2000). A possible way for the endocannabinoid system to affect motor activity is to influence dopaminergic transmission by controlling dopamine release. However, there is no co-expression or co-localization (see Fig. 2) of cannabinoid CB_1 receptors with tyrosine hydroxylase, which is the rate-limiting biosynthetic enzyme for dopamine formation, in neurons in the basal ganglia (Herkenham et al., 1991a; Julian et al., in press; Tsou et al., 1998). This suggests that there is no direct control of endocannabinoids on dopaminergic neurons. In line with this hypothesis is that cannabinoid CB_1 receptor agonists and antagonists were unable to inhibit electrically evoked release of endogenous dopamine in the corpus striatum (Szabo et al., 1999). However, other studies have reported an increased release of dopamine and firing of dopaminergic neurons of the substantia nigra after activation of the cannabinoid CB_1 receptor (French et al., 1997; Malone and Taylor, 1999; Melis et al., 2000; Ng Cheong Ton et al., 1988; Wu and French, 2000) or a reduction in dopamine release (Cadogan et al., 1997). A trans-synaptic mechanism in both the indirect and direct pathway may explain these apparently contradictory findings. Inhibition of the indirect pathway may lead to a reduced activation of the substantia nigra, i.e., and hence a reduced negative output to its target nuclei (facilitation of movement). On the other hand, inhibition of the direct pathway may lead to indirect activation of the substantia nigra and hence to an increased inhibitory control (inhibition of movement and catalepsy), as well as to activation of dopaminergic neurons, thereby inducing release of dopamine in the striatum. For example, it has been suggested that the excitatory corticostriatal projections contain presynaptic cannabinoid CB_1 receptors and their activation leads to a reduction of glutamate release by inhibition of N-type Ca^{2+} channels and by a Ca^{2+}-independent mechanism (Gerdeman and Lovinger, 2001; Gerdeman et al., 2002; Huang et al., 2001). Since corticostriatal afferents converge on medium spiny GABAergic neurons, which project to nuclei of both the indirect (globus pallidus externa) and the direct pathway (globus pallidus interna and substantia nigra), a reduction of their glutamatergic input may lead to inhibition of these pathways. In the indirect pathway, based on theoretical considerations, one would expect a cannabinoid CB_1 receptor-mediated reduction in GABAergic transmission in the external globus pallidus, which would lead to a reduced activation of the substantia nigra. However, experimental evidence points towards a facilitation of GABAergic transmission in the globus pallidus, due to a reduced GABA-uptake (Glass et al., 1997; Maneuf et al., 1996a,b; Pertwee, 1988). If this is the case in vivo, this would result in a reduced inhibition of the subthalamic nucleus and activation of the substantia nigra, thus explaining cannabinoid-induced catalepsy. Interestingly, dopamine depletion from the striatum was accompanied by an increase of 2-arachidonoylglycerol levels in the external layer of the globus pallidus, with concurrent blockade of movement, both of which were counteracted by dopamine D_2 receptor stimulation (Di Marzo et al., 2000d). On the other hand, the glutamatergic input of the substantia nigra by efferents of the subthalamus nucleus can be reduced via activation of presynaptic cannabinoid CB_1 receptors (Sanudo-Pena and Walker, 1997; Szabo et al., 2000). This would lead to a reduced activation of dopaminergic neurons and hence to a reduction of dopamine release in the striatum (Cadogan et al., 1997). The inhibition of the substantia nigra pars reticulata will result in a facilitation of movement. With respect to the direct pathway, it has been shown that GABAergic striatonigral projections are reciprocally ending with dopaminergic projections (Julian et al., in press). Since cannabinoid CB_1 receptors are located on these inhibitory GABAergic striatal efferent terminals, activation of these receptors may indirectly activate neurons from the globus pallidus interna and substantia nigra by inhibition of GABA release (Wallmichrath and Szabo, 2002) and hence lead to a further inhibition of its target nuclei and to release of dopamine (French et al., 1997; Malone and Taylor, 1999; Melis et al., 2000; Ng Cheong Ton et al., 1988; Wu and French, 2000), which might serve as a feedback mechanism for the restoration of normal movement. In keeping with this hypothesis is that stimulation of CB_1 receptors increased catalepsy produced by

administration of dopamine receptor antagonists (Anderson et al., 1996) and reduced the anti-Parkinson-like actions of dopamine D_2 receptor agonists (Maneuf et al., 1997). Furthermore, the cannabinoid CB_1 receptor antagonist *N*-(piperidin-1-yl)-5-(4-chloro-phenyl)-1-(2,4-dichloro-phenyl)-4-methyl-1*H*-pyrazole-3-carboxamide HCl (SR141716A) increased the efficacy of anti-Parkinson-like effects of dopamine receptor agonists in these rats (Di Marzo et al., 2000d).

Recently, it was suggested that endocannabinoids could be released post-synaptically from medium spiny neurons and serve as retrograde messengers in the dorsal striatum to induce long-term depression of the glutamatergic input by activation of the presynaptic cannabinoid CB_1 receptor (Gerdeman et al., 2002). Neural activity and stimulation of dopamine D_2-like, but not D_1-like, resulted in increased anandamide release in the striatum of freely moving rats (Giuffrida et al., 1999). This would imply that the medium spiny neurons of the indirect pathway are 'cannabinergic', but it does not exclude the possibility that medium spiny neurons from the direct pathway are also able to produce endocannabinoids. On the other hand, blockade of dopamine release from nigrostriatal efferents, as in 6-hydroxydopamine- or reserpine-treated rats, causes an increase in the striatal levels of endocannabinoids (Gubellini et al., 2002; Di Marzo et al., 2000d) (see below), thus suggesting that endocannabioid levels are also under negative control by dopamine in the dorsal striatum. These two sets of data are compatible with each other if one assumes that dopamine D_2-like receptor stimulation enhances, whereas dopamine D_1-like receptor stimulation reduces endocannabinoid levels in the striatum. When striatal dopamine release is impaired, the inhibitory effect, rather than the stimulatory one, is unmasked. On the contrary, when dopamine D_2-like receptors are selectively stimulated under physiological conditions, a stimulatory action can be revealed. Indeed, it has recently been shown that dopamine D_1 receptor activation by a selective agonist decreased anandamide concentrations in the limbic forebrain of mice, whereas dopamine D_1 receptor antagonists and dopamine D_2 receptor-like agonists, the latter only at a low dose, increased anandamide concentrations (Patel et al., 2003). The hypothesis of opposite effects of dopamine D_1-like and D_2-like receptor stimulation on endocannabinoid levels, however, is not consistent with the previous finding that stimulation of cAMP-protein kinase A signaling, rather than its inhibition, leads to enhanced anandamide formation in neurons (Cadas et al., 1996). On the other hand, at the moment, it is not clear whether the increased anandamide levels after dopamine D_2-like receptor stimulation results from increased biosynthesis or from an inhibited degradation, and different effects on the two metabolic branches may, for example, explain why in reserpine-treated rats stimulation of D_2-like receptor leads to a *decrease* of endocannabinoid levels in the external layer of the globus pallidus but not in the striatum (Di Marzo et al., 2000c) (see also below). It has been also proposed that endocannabinoid biosynthesis in the striatum acts as form of coincidence detector, stimulated by the coordinated actions of glutamate and dopamine (Gerdeman et al., 2003).

It was suggested that up-regulation of endocannabinoid signaling after dopamine D_2-like receptor stimulation represents a negative feedback mechanism for dopaminergic transmission in the striatum (Giuffrida et al., 1999). Pretreatment with an anandamide uptake inhibitor reduced the dopamine D_2 receptor-mediated motor behavior in rats, which was instead enhanced by a selective cannabinoid CB_1 receptor antagonist (Beltramo et al., 2000; Giuffrida et al., 1999). In keeping with these finding is that agonists of dopamine D_2-like receptors directly applied to rat basal ganglia counteract the behavioral responses of locally injected cannabinoid CB_1 receptor agonists (Sanudo-Pena et al., 1996, 1998; Sanudo-Pena and Walker, 1998). At the moment, the neurochemical mechanism of this feed-back mechanism is not clear. The endocannabinoid-induced reduction in dopamine D_2 receptor-mediated motor behavior might rely on the capability of the endocannabinoid system to inhibit the indirect pathway as discussed above, but also dopamine receptor-mediated cellular responses might be modified by the endocannabinoid system. A prerequisite for this latter type of interaction is the co-localization of post-synaptic cannabinoid CB_1 receptors with dopamine receptors. Indeed, cannabinoid CB_1 receptor mRNA has been found to be co-expressed with dopamine D_1 and D_2 receptor mRNA in striatum (Hermann et al., 2002). Selective destruction of striatal efferents resulted in loss of dopamine D_1 and D_2 and cannabinoid CB_1 protein in the striatum and topographic losses of cannabinoid CB_1 and dopamine D_1 receptors in globus pallidus, entopeduncular nucleus and substantia nigra pars reticulata (Herkenham et al., 1991a). This suggests that cannabinoid CB_1 receptors and dopamine D_1 receptors are colocalized in striatopallidal and striatonigral pathways (i.e., direct pathways). Indeed, converging functional cellular interactions between the dopamine D_1 receptor and the cannabinoid CB_1 receptor have been observed. Cannabinoid CB_1 receptor stimulation resulted in the inhibition of dopamine D_1 receptor-induced formation of cAMP within rat and monkey striatum (Bidaut-Russell and Howlett, 1991; Meschler and Howlett, 2001). Thus, the inhibitory effect of post-synaptic cannabinoid CB_1 receptors on dopamine D_1 receptor signaling may reduce the activation of the direct pathway and act in concert with a presynaptic, cannabinoid CB_1 receptor-mediated reduced glutamatergic input of the striatal medium spiny neurons.

Since both cannabinoid CB_1 receptor and the dopamine D_2 receptors couple to the same cellular effector systems (Meschler and Howlett, 2001), it is less likely that the endocannabinoid system interferes with dopaminergic D_2 signaling. In fact, converging facilitatory effects between the dopaminergic signalling and the endocannabinoid system have been found in vivo in nonhuman primates. Dopamine D_2 receptor agonists reduced the threshold dose for canna-

binoid CB_1 receptor agonist-induced sedation in monkeys (Meschler et al., 2000). Concurrent stimulation of cannabinoid CB_1 receptors and dopamine D_2 receptors in primary striatal cultures has been found to result in an increase of cAMP accumulation, whereas activation of either receptor alone led to an inhibition of forskolin-induced cAMP formation (Glass and Felder, 1997). If this also occurs in vivo, the simultaneous activation may lead to a stimulation of the indirect pathway, i.e., enhanced GABAergic signaling in the globus pallidus, reduced activation of the subthalamic nucleus, which results in further inhibition of the substantia nigra, and hence to facilitated motor behavior.

In summary, cannabinergic signalling in the direct pathway may underlie the motor depressant effect of cannabinoids and may lead to release of dopamine, which can act, possibly via dopamine D_1-like receptors, as a negative feedback mechanism to counteract the motor inhibition of cannabinoids. On the other hand, dopaminergic signalling via dopamine D_2-like receptors in the indirect pathway may lead to up-regulation of cannabinergic signalling in the striatum, which might represent a negative feedback on striatal dopaminergic signalling. In addition, dopamine D_2 receptor activation of medium spiny neurons may lead to a down-regulation of cannabinergic signalling in the external layer of the globus pallidus (Di Marzo et al., 2000c), which represents either a negative or positive feedback mechanism via inhibition of GABA release or uptake, respectively.

3.1. Parkinson's disease

Parkinson's disease is a chronic, progressive disorder of late life, which is characterized by rigidity, unintentional tremor and bradykinesia. There is a selective degeneration of dopaminergic neurons in the nigrostriatal pathway, which is thought to be related to their particular vulnerability to oxidative stress. The resulting dopamine deficiency in the striatum leads to imbalances in the basal ganglia physiology, which may include (i) an enhanced corticostriatal glutamatergic drive, and (ii) an overactivation of the indirect pathway, i.e., increased GABAergic transmission in the globus pallidus (Blandini et al., 2000; Calabresi et al., 1996).

The involvement of the endocannabinoid system in neurodegenerative diseases of the basal ganglia has received much attention (for reviews, see Van der Stelt et al., 2002; Fernandez-Ruiz et al., 2002; Brotchie, 2003). With respect to Parkinson's disease, it has been suggested that changes in the endocannabinoid system may participate in symptom generation or are part of a compensatory mechanism to counteract the unbalance in basal ganglia physiology. For example, it has been shown that the enhanced corticostriatal glutamatergic transmission in dopamine depleted animals (induced by 6-hydroxydopamine) could be attenuated by stimulation of the endocannabinoid system, i.e., by application of the cannabinoid CB_1 receptor agonists, anandamide uptake inhibitors and fatty acid amide hydrolase inhibitors (Gubellini et al., 2002). Increased striatal levels of anandamide, but not of 2-arachidonoylglycerol, were observed in the dopamine depleted animals, which might seem to contradict the dopamine-induced formation of anandamide (Giuffrida et al., 1999). However, elevated anandamide levels in dopamine-depleted striatum were assumed to be caused by a down-regulation of its metabolism, i.e., an abnormal down-regulation of anandamide membrane transport and fatty acid amide hydrolase activity (Gubellini et al., 2002). It was suggested that the down-regulation of the proteins of anandamide inactivation pathway were a compensatory, albeit partly unsuccessful, mechanism to reduce the increased cortical glutamatergic transmission. Consequently, inhibition of anandamide hydrolysis might represent a possible target to decrease the glutamatergic drive in Parkinson's disease (Gubellini et al., 2002). Dopamine replacement therapy with L-3,4-dihydroxyphenylalanine (L-DOPA) reversed the abnormalities in the endocannabinoid system in this animal model (Maccarrone et al., 2003). In keeping with these findings is that in most animal models of Parkinson's disease in which dopamine is depleted from the striatum by 6-hydroxydopamine or by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, there is an up-regulation of striatal cannabinoid CB_1 mRNA, which returned to normal levels by L-DOPA treatment (Lastres-Becker et al., 2001a; Mailleux and Vanderhaeghen, 1993; Romero et al., 2000). This would suggest that cannabinoid CB_1 receptor expression is under negative control of dopamine transmission and its up-regulation is an attempt to normalize GABAergic signalling in the globus pallidus. However, it should be noted that changed CB_1 receptor mRNA levels in cell bodies of striatal efferent neurons are not always accompanied by changes in cannabinoid CB_1 receptor binding capacity and activation of intracellular signal transduction mechanisms (Gubellini et al., 2002; Romero et al., 2000). Furthermore, differently from the 6-hydroxydopamine model, a down-regulation of striatal cannabinoid CB_1 mRNA levels in the reserpine model of Parkinson's disease was instead found, which was assumed to be secondary to increased endocannabinoid levels (Silverdale et al., 2001).

It is thought that an enhanced GABAergic transmission in the globus pallidus, i.e., the indirect pathway, might contribute to the symptoms in Parkinson's disease (Brotchie, 2003). As stated above, the increased 2-arachidonoylglycerol levels in the globus pallidus of reserpine-treated rats (Di Marzo et al., 2000d), might contribute to increasing GABAergic transmission in the globus pallidus by inhibiting GABA uptake (Maneuf et al., 1996a,b). Thus, it was suggested that the enhanced endocannabinoid levels in the globus pallidus contribute in part to the pathophysiology of parkinsonian symptom generation and that selective cannabinoid CB_1 receptor antagonists might be useful for therapeutic treatment (Di Marzo et al., 2000d). In a recent study with non-human primates, however, SR141716A failed to

alleviate the parkinson-like symptoms (Meschler et al., 2000, 2001), which might suggest that the basal ganglia physiology in primates is different from rodents and or that different methods to deplete dopamine from the striatum results in diverging result.

Finally, the exact role of the endocannabinoid system in the etiology of L-DOPA-induced dyskinesia has only been poorly investigated. In this case, one would expect that endocannabinoid levels in the basal ganglia might be lower than in healthy subjects, thus explaining in part the uncontrolled movements typical of this Parkinson's disease consequence. Indeed, changes induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine treatment in marmosets in both cannabinoid CB_1 receptor levels and signaling were reversed by L-DOPA (Lastres-Becker et al., 2001a), which also reverted the changes in striatal endocannabinoid levels found in the 6-hydroxydopamine model in rats (Maccarrone et al., 2003). This indicates that, indeed, endocannabinoids are enhanced during Parkinson's disease as a response to counteract the neurochemical and neurological unbalance, and perhaps the neuronal damage, caused by this disorder. Much in the same way that this over-production might also cause some side effects, such as motor impairment, its correction by L-DOPA treatment might counteract the beneficial action of endocannabinoids during L-DOPA-induced dyskinesia. In fact, this latter disorder appears to benefit significantly from CB_1 receptor stimulation by agonists both in animal models and humans (Fox et al., 2002; Segovia et al., 2003; Sieradzan et al., 2001).

3.2. Huntington's disease

Huntington's disease is an autosomal-dominant disorder in which a mutation in a gene located on chromosome 4 (*4p16.3*), its product being huntingtin, is responsible for an unstable expansion in a poly-CAG repeat, and hence for the development of progressive involuntary choreiform movements and cognitive deficits. The functions of the normal and mutated protein are still unknown, but expression of the mutated huntingtin in early and pre-symptomatic cases of Huntington's disease leads to selective loss of GABA/enkephalin-containing medium spiny neurons projecting from the striatum to the globus pallidus externa (indirect pathway) and GABA/substance P-containing striatal neurons projecting to the substantia nigra (direct pathway), while there is a relative sparing of GABA/substance P-neurons projecting to the globus pallidus interna (direct pathway). However, in severe grades of Huntington's disease, all striatal projections show extensive loss. The progression of the disease and the age of onset are dependent on the number of the CAG-copy numbers (Feigin and Zgaljardic, 2002).

In postmortem brains of patients with Huntington's disease, a relative loss of cannabinoid CB_1 and dopamine receptors was observed through several grades of pathology in the globus pallidus and substantia nigra (Glass et al., 2000). Within the globus pallidus cannabinoid CB_1 receptor binding was highly decreased in the globus pallidus externa in the early Huntington's disease cases, which exceeded the loss of binding in the globus pallidus interna. In the latter region, the loss of cannabinoid CB_1 receptor preceded the terminal atrophy, because it occurred before the loss of co-localized dopamine receptors was observed. Interestingly, a cell-specific and time-dependent regulation of the cannabinoid CB_1 receptor mRNA as a result of the expression of mutated huntingtin has recently been observed in two different strains of transgenic mouse models of Huntington's disease (Denovan-Wright and Robertson, 2000; Lastres-Becker et al., 2002a). It was shown that, prior to the development of either Huntington's disease phenotype or neuronal degeneration, cannabinoid CB_1 receptor mRNA was down-regulated in the lateral striatum, in cortical regions and in a subset of hippocampal neurons in the brain of R6/2 mice (Denovan-Wright and Robertson, 2000). In HD94 mice, which over-express a smaller poly-CAG repeat (94 copies) than the R6/2-mice (>115 copies), cannabinoid CB_1 receptor mRNA was also decreased in the striatum, but not in the cerebral cortex and hippocampus (Lastres-Becker et al., 2002a). This transcriptional effect was accompanied by a reduction in receptor levels, as measured with (−)-*cis*-3-(2-hydroxy-4-(1,1-dimethylheptyl)phenyl)-*trans*-4-(3-hydroxypropyl)cyclohexanol ([^{3}H]CP55,940) in the striatum and in its projection areas such as the globus pallidus, entopeduncular nucleus and substantia nigra pars reticulata. The decrease of CB_1 receptor levels was paralleled by a decrease in proenkephalin mRNA, but not in substance P mRNA levels (Lastres-Becker et al., 2002a). The efficacy of receptor activation was only significantly reduced in the globus pallidus, suggesting that there is a difference in vulnerability in the two striatal efferent pathways.

By contrast, in rat models of Huntington's disease where 3-nitropropionic acid, a mitochondrial neurotoxin, is used to produce striatal lesions, the loss of cannabinoid CB_1 receptors was associated with neuronal death (Lastres-Becker et al., 2001b; Page et al., 2000). Cannabinoid CB_1 receptor binding sites were lost, and the cannabinoid CB_1 receptor-mediated activation of GTP-binding proteins were reduced in the selectively damaged striatal GABAergic efferent neurons (Lastres-Becker et al., 2001b). Enkephalin- and substance P-containing neurons were equally affected. These changes were accompanied by a decrease of anandamide and 2-arachidonoylglycerol levels in the striatum, whereas normal endocannabinoid levels were found in the non-lesioned cerebral cortex (Lastres-Becker et al., 2001b). At the moment, it is unclear to which extent these changes contribute to the symptoms of Huntington's disease, or whether they are side effects of toxin-induced destruction of striatal GABAergic neurons (Lastres-Becker et al., 2001b).

It is speculated that the early down-regulation of cannabinoid receptors and their endogenous ligands is a compensatory mechanism in Huntington's disease, because it might increase GABA release, which could counteract the initial

loss of GABAergic neurons (Glass, 2001). This hypothesis has not been verified yet, but, if valid, it would imply that blockade of cannabinoid CB_1 receptor function might be able to slow down the progression of Huntington's disease. On the other hand, it may also be speculated that the early loss of cannabinoid CB_1 signaling in the striatum, while possibly contributing to the hyperkinesia typical of Huntington's disease, results in an imbalanced glutamatergic transmission, thereby eliciting excitotoxicity and subsequent neurodegeneration. As yet, no cannabinoid CB_1 receptor agonists have been tested to prevent neurodegeneration in animal models of Huntington's disease. It was shown that the anandamide uptake inhibitor *N*-(4-hydroxyphenyl)arachidonylethanolamine (AM404) could attenuate motor disturbances and neurochemical unbalances in the early phase of hyperactivity in 3-nitropropionic acid-treated rats by restoring GABA and dopamine transmission (Lastres-Becker et al., 2002b). However, in a follow-up study, this effect was shown to be due to stimulation of vanilloid VR1 receptors, for which AM404 is a rather potent agonist (Zygmunt et al., 1999; Lastres-Becker et al., 2003). The anti-hyperkinetic and GABA-restoring effects of AM404 were counteracted by the vanilloid VR1 receptor antagonist capsazepine (Lastres-Becker et al., 2003), but not by the CB_1 antagonist SR141716A. They were mimicked by the prototypical vanilloid VR1 receptor agonist capsaicin, which was previously found to reduce locomotion by acting at vanilloid receptors in the rat (Di Marzo et al., 2001b). Another anandamide uptake inhibitor, VDM-11, as well as capsazepine and SR141716A were inactive against hyperkinesia in the 3-nitropropionic acid model, thus arguing against a tonic beneficial role of endocannabinoids and endovanilloids in this model. The authors suggested that the 3-nitropropionic acid-induced lesion spares vanilloid VR1 receptor, rather than cannabinoid CB_1, receptor-expressing neurons. If this phenomenon also occurs in other Huntington's disease models, it might open the way to the use of "hybrid" cannabinoid CB_1/vanilloid VR1 receptor agonists against neurological and neurochemical dysfunctions in Huntington's disease.

4. Interactions between dopaminergic transmission and the endocannabinoid system in the mesocorticolimbic pathway

Dopaminergic neurons in the mesocorticolimbic pathway are mainly located in the A10 cell group of the ventral tegmental area and project topographically to limbic forebrain structures such as the prefrontal cortex and to the nucleus accumbens (Fig. 3). The nucleus accumbens, which is a part of the ventral striatum, also receives glutamatergic input from the prelimbic cortex. GABAergic interneurons within the ventral tegmental area and a long-loop feedback projection from GABAergic medium spiny neurons in the nucleus accumbens provide tonic inhibition of A10 neurons.

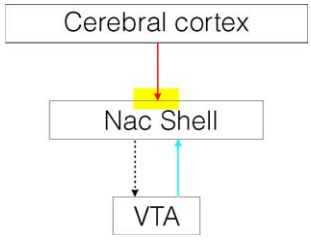

Fig. 3. Simplified scheme of the functional organization of the mesolimbic reward system. Dopaminergic neurons in the ventral tegmental area (VTA) project (blue arrow) to medium spiny neurons in the shell of the nucleus accumbens (NAc Shell). These medium spiny neurons receive also glutamatergic projections (red arrow) from various cerebral cortical areas and send a long-loop feedback with GABAergic projections (black arrow) to the ventral tegmental area. The cannabinoid CB_1 receptor (yellow box) is presynaptically expressed on cortical efferents in the shell of the nucleus accumbens, which synapse with the medium spiny neurons. See text for a detailed description of the role of the endocannabinoid system in the nucleus accumbens.

Activation of the mesocorticolimbic pathway is thought to have an important function in the acquisition of behavior which is reinforced by natural rewarding stimuli and drugs of abuse. Dopaminergic transmission in the nucleus accumbens is preferentially increased by natural rewards such as food, water and sex under conditions of deprivation, and in normal conditions, novelty seems to be an important determinant of activation of the dopaminergic neurons (Spanagel and Weiss, 1999).

Like in the dopaminergic nigrostriatal pathway, there is no expression of cannabinoid CB_1 receptors mRNA nor protein in dopaminergic neurons in the ventral tegmental area, which seems to rule out a direct control of endocannabinoids over dopamine release in the nucleus accumbens and prefrontal cortex (Herkenham et al., 1991b; Matsuda et al., 1993; Patel and Hillard, 2003; Szabo et al., 1999; Tsou et al., 1998). Nevertheless, increased dopamine release and firing of dopaminergic neurons in these structures have been found after systemic administration of the cannabinoid CB_1 receptor agonists, which could be blocked by the selective cannabinoid CB_1 receptor antagonists (Chen et al., 1990; French, 1997; Gessa et al., 1998; Tanda et al., 1997, 1999; Wu and French, 2000). This suggests that the endocannabinoid system may exert a trans-synaptic control over the dopaminergic neurons in the mesocorticolimbic pathway. However, the absence of cannabinoid CB_1 receptor protein in the ventral tegmental area seems to exclude the possibility that activation of presynaptic cannabinoid CB_1 receptors directly inhibit GABA release from interneurons or from projection terminals of the medium spiny neurons of the nucleus accumbens (Herkenham et al., 1991b; Matsuda et al., 1993; Patel and Hillard, 2003; Tsou et al., 1998). Instead, the cannabinoid CB_1 receptor may modulate GABAergic and glutamatergic neurotransmission in the nucleus accumbens (Hoffman and Lupica, 2001; Manzoni and Bockaert, 2001; Robbe et al., 2001, 2002). It has been demonstrated that cannabinoid CB_1 receptors are present at

the glutamatergic synapses in the nucleus accumbens, which make contact with the long-loop feedback GABAergic medium spiny neurons (Robbe et al., 2001). The glutamatergic afferents to the nucleus accumbens control the firing of the GABAergic medium spiny neurons, which, in turn, inhibit the dopaminergic neurons in the ventral tegmental area. Activation of the presynaptic cannabinoid CB_1 receptor at the cortical glutamatergic afferents has been shown to strongly reduce excitatory transmission (Pistis et al., 2002; Robbe et al., 2001, 2002); only one study showed a slight reduction in excitatory transmission that might have been due to a post-synaptic effect (Hoffman and Lupica, 2001). Thus, activation of cannabinoid CB_1 receptors may disinhibit dopaminergic A10 cells, increase their firing rate and trigger dopamine release via the reduction of excitatory transmission in the nucleus accumbens and the subsequent inhibition of GABAergic signaling from medium spiny neurons (Fig. 3).

Recently, it was proposed that the endocannabinoid system could be engaged in a negative feedback loop reducing glutamatergic synapses strength during sustained cortical activation of the nucleus accumbens. It was shown that endocannabinoids could be released from medium spiny neurons in the nucleus accumbens and travel 'backwards' to the presynaptic cannabinoid CB_1 receptor to induce long-term depression of the glutamatergic input (Robbe et al., 2002). Activation of post-synaptic metabotropic glutamate receptors and intracellular Ca^{2+} stores were shown to be necessary to elicit long-term depression. In contrast to the endocannabinoid-dependent long-term depression of the dorsal striatum, activation of dopamine receptors does not seem to be involved in long-term depression in the nucleus accumbens (ventral striatum) (Gerdeman et al., 2002; Robbe et al., 2002). It would be interesting to understand how this finding relates to remodeling of synaptic plasticity by drugs of abuse, including marijuana itself (for a recent review, see Gerdeman et al., 2003).

4.1. Drug addiction

Drug addiction can be viewed as a psychiatric disorder in which neuronal mechanisms underlying learning and memory are malfunctioning (Berke and Hyman, 2000; Gerdeman et al., 2003). A common feature for drugs of abuse, such as amphetamine, cocaine and opioids like morphine and heroin, is their activation of the mesolimbic dopaminergic neurons. Both amphetamine and cocaine increase dopamine concentrations in the nucleus accumbens by blocking its uptake through a transporter, whereas opioids may activate μ- or δ-opioid receptors on GABAergic interneurons in the ventral tegmental area, thereby producing disinhibition and increased firing of the dopaminergic neurons. It is thought that chronic drug use induces long-term neuroadaptive changes within the mesolimbic dopaminergic system and leads to desentitisation (tolerance) of the neural mechanisms that mediate reward, and sensititisation to the behavioral actions of these drugs. This might contribute to compulsive drug-seeking behavior and relapse (Spanagel and Weiss, 1999).

The ability of cannabinoids to indirectly activate the same mesolimbic dopaminergic pathways as other drugs of abuse and their similar actions in behavioral models of addiction have aroused a debate on the potential of marijuana to induce dependence in humans (for reviews, see Gardner and Vorel, 1998; Maldonado, 2002; Maldonado and Rodriguez de Fonseca, 2002). In summary, drug-discrimination studies have shown that a selective cross discrimination between natural and synthetic cannabinoids exist, but not with non-cannabinomimetic drugs (Wiley et al., 1995). Following chronic cannabinoid administration, including anandamide, tolerance in various behavioral paradigms develops and a withdrawal syndrome can be precipitated with selective cannabinoid CB_1 receptor antagonists (Costa et al., 2000; Rodriguez de Fonseca et al., 1997; Rubino et al., 1997), which is accompanied by a reduced mesolimbic dopaminergic transmission (Diana et al., 1998; Tanda et al., 1999). A behavioral sensitisation to amphetamine and heroin has also been demonstrated in cannabinoid-tolerant rats (Gorriti et al., 1999; Lamarque et al., 2001; Muschamp and Siviy, 2002). Anandamide and 2-arachidonoylglycerol levels were decreased in the striatum of THC-tolerant rats, whereas only anandamide levels were increased in the limbic forebrain including the nucleus accumbens, which indicated that the regulation of the biosynthesis of endocannabinoids is region-dependent (Di Marzo et al., 2000b). In most reported studies, chronic cannabinoid treatment leads to a region-dependent reduction of cannabinoid CB_1 receptor expression and to a less efficient coupling to its effector systems, thereby providing a rational for cannabinoid-induced tolerance (Fan et al., 1996; Romero et al., 1995; Rubino et al., 1998, 2000a,b). It was suggested that the down-regulation of the cannabinoid CB_1 receptor in cannabinoid-tolerant rats relieved the dopaminergic system from its endogenous inhibition by the endocannabinoid system, thereby making it more sensitive towards the effects of amphetamine (Gorriti et al., 1999). In accordance with this hypothesis, it has recently been shown that chronic THC or WIN55,212-2 treatment in rats resulted in a reduced sensitivity of glutamatergic and GABAergic synapses to the inhibitory effects of cannabinoids, and that endocannabinoid-induced LTD in the nucleus accumbens was abolished, thereby demonstrating that long-term exposure to cannabinoids blocks synaptic plasticity (Hoffman et al., 2003).

The rewarding properties of cannabinoids can be detected under special conditions, which are different from other drugs of abuse, in behavioral paradigms such as intracranial self-stimulation, self-administration and conditioned place preference (Gardner et al., 1988; Martellotta et al., 1998; Tanda et al., 2000; Valjent and Maldonado, 2000). However, in the latter paradigm, THC produces in naive

animals also aversive responses (Elsmore and Fletcher, 1972), and THC self-administration can only be demonstrated in animals with a cocaine self-administration history (Tanda et al., 2000). Using a more potent cannabinoid CB_1 receptor agonist, 2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)-pyrrolo-1,4-benzoxazin-6-yl-1-naphthalenylmethanone (WIN55,212-2), other authors have been able to demonstrate self-administration in rats and mice (Fattore et al., 2001; Martellotta et al., 1998). However, non-CB_1 and non-CB_2 cannabinoid receptors for this substance have been suggested to exist in rodent brain (Breivogel et al., 2001).

Cannabinoids might exert part of their reinforcing effects through the endogenous opioid system (Maldonado and Rodriguez de Fonseca, 2002; Manzanares et al., 1999). For example, cannabinoid CB_1 receptor-mediated release of dopamine in the nucleus accumbens could be blocked by naloxone, an opioid receptor antagonist (Tanda et al., 1997). Furthermore, the THC withdrawal syndrome could be precipitated with naloxone (Navarro et al., 1998) and is significantly reduced in mice lacking the μ-opioid receptor (Lichtman et al., 2001) or in mice lacking proenkephalin, i.e., the precursor for the endogenous activator for this receptor (Valverde et al., 2000). Also, THC-induced conditioned place preference is suppressed in μ-opioid receptor knock-out mice (Ghozland et al., 2002) and THC-induced self-administration can be blocked by μ-opioid receptor antagonists (Fattore et al., 2001; Navarro et al., 1998). The neurochemical mechanism of the interaction between the endocannabinoid and opioid systems has not been elucidated, but might involve cannabinoid-induced synthesis and release of endogenous opioids or converging signal transduction pathways if the receptors are co-expressed (Manzanares et al., 1999). The interaction between the endocannabinoid and μ-opioid system seems to be mutual. The tolerance, withdrawal syndrome and conditioned place preference induced by morphine are reduced in cannabinoid CB_1 knockout mice (Ledent et al., 1999; Martin et al., 2000). However, the hypothesis that marijuana use might enhance the reinforcing properties of opioids warrants further research, because agonists of the cannabinoid CB_1 receptor, such as THC, *R*(−)-7-hydroxy-delta-6-tetrahydrocannabinol-dimethylheptyl (HU210), anandamide and 2-arachidonoyl, and AM404 (an anandamide uptake inhibitor) can also reduce morphine withdrawal symptoms (Del Arco et al., 2002; Hine et al., 1975; Vela et al., 1995; Yamaguchi et al., 2001). Furthermore, diverging and region-dependent effects in cannabinoid CB_1 receptor expression upon chronic treatment with morphine have been found (Romero et al., 1998a) (see Viganó et al., 2003 for discussion). Indeed, chronic opiate administration to rats seems to impact on the elements of the endocannabinoid system in a different way from other substances of abuse, such as nicotine and ethanol. While the two latter substances (like THC) produce an *enhancement* of anandamide levels in the nucleus accumbens-containing limbic forebrain (Gonzalez et al., 2002b), as well as a cannabinoid CB_1 receptor-dependent release of dopamine in the nucleus accumbens (Cohen et al., 2002), a *reduction* in the coupling of the cannabinoid CB_1 receptor to its G-protein, accompanied by reduced levels of 2-arachidonoylglycerol, was recently described in the limbic forebrain of morphine-dependent rats (Vigano et al., 2003). These observations, as well as the lack of any effect of chronic cocaine treatment on both cannabinoid receptor levels/signalling and endocannabinoid amounts (Gonzalez et al., 2002a), might suggest that, rather than helping the brain to be predisposed to the consumption of heroin and cocaine, the endocannabinoid system might instead selectively reinforce the effects of milder hedonic stimuli and help the brain coping with the consequences of the self-administration of "strong" substance of abuse.

The endocannabinoid system seems to be involved instead in the relapse mechanism to heroin and cocaine seeking (De Vries et al., 2001, 2003). The synthetic cannabinoid HU-210 provoked relapse to drug seeking after a prolonged withdrawal period, while blockade of the cannabinoid CB_1 receptor attenuated the relapse induced by re-exposure to drug-associated cues or the drug itself. It was hypothesized that the cocaine-priming-induced reinstatement was induced by dopamine-induced formation of endocannabinoids, rather than an endocannabinoid-induced release of dopamine, because blockade of opioid receptors did not have any effect on the relapse, whereas it could block cannabinoid-induced release of dopamine (De Vries et al., 2001). Two synthetic cannabinoid CB_1 receptor agonists, but not THC, reinstated consumption of heroin in rats previously trained to self-administer the drug (Fattore et al., 2003). Also in this case, the cannabinoid CB_1 antagonist SR141716A counteracted the relapse of heroin self-administration induced by a cue of heroin. Interestingly, a natural single nucleotide polymorphism (SNP) that leads to a missense mutation in human fatty acid amide hydrolase, thereby enhancing its sensitivity to proteolytic degradation, was associated with drug abuse in humans (Sipe et al., 2002). Could it be that these patients are more vulnerable to relapse, due to a reduced degradation of anandamide? At any rate, the data suggest that selective cannabinoid CB_1 receptor antagonists might be useful to prevent relapse.

4.2. Hyperphagia and anorexia

The mesolimbic dopaminergic pathway has also been suggested to be involved in the rewarding properties of food. It has been shown that under conditions of food restriction, dopamine will be released in the nucleus accumbens after an incentive stimulus (Spanagel and Weiss, 1999). Interestingly, food-deprived rats were found to exhibit enhanced levels of endocannabinoids, not only in the hypothalamus, which controls the appetitive aspects of food intake, but also in the limbic forebrain (Kirkham et al., 2002). Furthermore, in the same study, it was found

that injection of 2-arachidonoylglycerol into the nucleus accumbens induces food intake in rats. These findings are in agreement with early observation that blockade of cannabinoid CB_1 receptors with SR141716A reduces not only the intake of "normal" food (Jamshidi and Taylor, 2001; Williams and Kirkham, 1999), but also, and possibly to a greater extent, the consumption of palatable foods and alcohol (Arnone et al., 1997; Simiand et al., 1998). Furthermore, a recent study showed that endocannabinoids increase sucrose licking behavior in rats (Higgs et al., 2003). In rats with diet-induced obesity, which is regarded as one of the animal models of obesity most relevant to the human condition, a down-regulation of cannabinoid CB_1 receptors, particularly in the nucleus accumbens, was observed (Harrold et al., 2002). This alteration was suggested to be due to changes in endocannabinoid levels in this area. Hence, both food deprivation and excessive food consumption seem to control endocannabinoid signalling in the nucleus accumbens, and this phenomenon might be related to the possible dopamine-related reinforcing effects of the endocannabinoid system on the rewarding properties of hedonic stimuli, mentioned above. The recent finding of a hypersensitising effect by cannabinoid CB_1 receptor stimulation on orexin 1 receptor (Hilairet et al., 2003) is interesting in view of the excitation of ventral tegmental area A10 dopaminergic neurons by orexins (Korotkova et al., 2003), and may provide another mechanism for the indirect stimulation of dopaminergic signalling by endocannabinoids in the nucleus accumbens.

Dopaminergic cells are also located in the arcuate nucleus of the hypothalamus (A12 and A14 cell groups) along the wall of the third ventricle. Cannabinoid CB_1 receptors have also been found in this cell group (Romero et al., 1998b). Therefore, although no thorough study has been performed to investigate this hypothesis, it is possible that endocannabinoids owe part of their food intake stimulatory actions to direct or indirect effects on arcuate nucleus dopaminergic signaling. At any rate, it is now well established that: (1) enhanced hypothalamic anandamide and, particularly, 2-arachidonoylglycerol levels accompany food deprivation in both rats and mice as a consequence of reduced circulating levels of leptin, which down-regulates endocannabinoid biosynthesis, and (2) endocannabinoids and CB_1 receptors participate to inducing food intake in food-deprived mice (Di Marzo et al., 2001a). Interestingly, the decrease in hypothalamic dopamine levels that follows diet restriction can be restored by anandamide at the same low doses that induce food intake in mice (Hao et al., 2000).

Much in the same way that endocannabinoids seem to be necessary to reinforce both the appetitive and hedonic aspects of food intake, a malfunctioning endocannabinoid system may contribute to psychiatric disorders such as anorexia nervosa. No study has been reported yet on this attractive possibility, but the widespread use of cannabis and dronabinol as appetite stimulants in AIDS or cancer patients suggests that administration of these drugs might represent a possible attempt to compensate for an impaired endocannabinoid system during anorexic states.

4.3. Schizophrenia

Schizophrenia is a psychiatric disorder that affects almost 1% of the human population worldwide. Schizophrenia is characterized by psychotic periods with positive symptoms such as memory disturbances, delusions and hallucinations, which are separated by periods with negative symptoms, such as socially isolated, low emotional arousal, low social drive and lack of attention span. Schizophrenia appears to be a polygenic neurodevelopmental disorder, for which a biochemical basis has not yet been identified. At the moment, it is thought that hyperactivity of dopamine neurotransmission in the mesencephalic projections to the nucleus accumbens may be contributing to the psychotic (positive) symptoms, whereas hypodopaminergic and hypoglutamatergic transmission in the prefrontal cortex might be related to the negative symptoms (Lewis and Levitt, 2002; Lewis and Lieberman, 2000; Thaker and Carpenter, 2001). It has been postulated that a subgroup of schizophrenic syndromes may be pathogenetically related to a functional disturbance of the endogenous cannabinoid system, i.e., the endocannabinoid hypothesis of schizophrenia (Emrich et al., 1997). It was suggested that an over-activity of the endocannabinoid system may lead to hyperdopaminergic and hypoglutamatergic transmission, giving rise to both the positive and negative symptoms of the disorder. Indirect evidence for this hypothesis comes from several studies. For example, cannabis consumption has been associated with both an increased risk for the onset of schizophrenia and with a decreased therapeutic effectiveness of antipsychotics (Negrete and Gill, 1999). Cannabis use may induce a psychosis similar to that seen in schizophrenia (Negrete and Gill, 1999) and it also mimics the attentional deficits and perceptional illusions seen in schizophrenia (Leweke et al., 1999b). Recently, it was shown in a case study that cannabis use induces dopamine release in a schizophrenic patient, which was followed by worsening of psychotic symptoms (Voruganti et al., 2001). Alterations in the endocannabinoid system have also been associated with schizophrenia. The cannabinoid CB_1 receptor was up-regulated in the prefrontal cortex of patients with schizophrenia (Dean et al., 2001). Recently, a single nucleotide polymorphism in coding exon 1 of *CNR1* gene (which expresses the cannabinoid CB_1 receptor) has been associated with schizophrenia in a French Caucasian population, but this single base mutation was silent, i.e., no change in amino acid sequence (Leroy et al., 2001). In a Japanese population, the AAT triplet repeats in the 3′ flanking region of the *CNR1* gene were associated with schizophrenia, especially the hebephrenic subtype (Ujike et al., 2002), but not in a Chinese population (Tsai et al., 2001). Furthermore, anandamide, but not 2-arachidonoyl-

glycerol, concentrations in the cerebrospinal fluid of schizophrenic patients appear to be increased (Leweke et al., 1999a). At the moment, it is unclear whether these elevated levels of anandamide result from a homeostatic adjustment of the endogenous cannabinoid system to hyperdopaminergic transmission or are a direct cause of the psychosis by being responsible for hyperdopaminergic transmission. Preliminary studies indicate that this alteration of anandamide levels can be observed also in the blood of patients with schizophrenia (P. De Marchi, V. Di Marzo and L. De Petrocellis, submitted) (Fig. 4), and that treatment with neuroleptics corrects the psychotic signs together with this unbalance in endocannabinoid signalling, in both blood cells (P. De Marchi, V. Di Marzo and L. De Petrocellis, submitted) and the cerebrospinal fluid (Fegley et al., 2002). These observations, if confirmed by more comprehensive studies, would suggest that altered anandamide levels in schizophrenia are a consequence of the hyperactivity of dopamine signalling, which impacts on central as well as peripheral tissues, and are corrected following treatment with neuroleptic drugs.

5. Anxiety disorders

Anxiety disorders are characterized by an increased fearfulness towards threatening situations or for events that do not present a real danger. They can be subdivided into several types, including panic disorder, post-traumatic stress disorder, social phobia, obsessive–compulsive disorder or generalized anxiety disorder. Although anxiety is not identical to fear, it is closely linked to fear learning (Kent et al., 2002). The amygdala is the key brain structure in coordinating the fear response. It receives information from diverse brain areas such as the prefrontal cortex, hippocampus and thalamus and it sends its information to autonomic neuroendocrine and motor systems to generate a

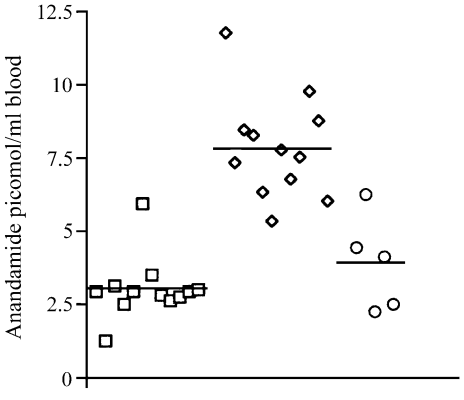

Fig. 4. Amounts of anandamide in the blood of either healthy humans (squares) or schizophrenic (rhombs) patients before and after treatment with olanzapine (circles). Anandamide levels were measured by isotope dilution–liquid chromatography–athmospheric pressure chemical ionization–mass spectrometric analysis of plasma + mononucleated cells. Details of this study will be published elsewhere.

behavioral response (Kent et al., 2002). Long-term potentiation plays a key role in establishing memory for fear conditioning. However, the extinction of aversive memories, rather than being exerted through the suppression of memory, represents the overlapping of a new mnemonic event over the previously acquired aversive memory. Recently, it has been demonstrated that the endocannabinoid system participates in generating these beneficial "overlapping memories" (Marsicano et al., 2002). First, it was shown that either acute or congenital blockade of cannabinoid CB_1 receptor signaling results in the inability of mice to extinguish an aversive memory acquired after a fear conditioning protocol, even after several days from conditioning. Furthermore, it was shown that the levels of endocannabinoids in the basolateral amygdala of wild-type mice are enhanced only in fear-conditioned animals and only after 24 h and upon re-exposure to the conditioning stimulus. It was also shown that this effect of endocannabinoids is probably due to them causing a long-term depression of inhibitory post-synaptic currents in interneurons of the basolateral amygdala, with possible disinhibition of the primary neurons of the basolateral amygdala, and formation of the active mnemonic processes responsible for extinction of the aversive memory. Clearly, if this neural mechanism is responsible for the purported antiphobic activity of cannabis, this has more to do with modulation of the GABAergic rather than the dopaminergic system, even though recent evidence points to a role of dopamine D_2 receptors in the inhibition of the extinction of aversive memories (Nader and LeDoux, 1999).

While data exist on both anxiolytic (Berrendero and Maldonado, 2002) and anxiogenic (Arevalo et al., 2001; Marin et al., 2003) effects of cannabinoids in rodents, the effects of endocannabinoids in animal models of anxiety have not been studied. Also, the cannabinoid CB_1 receptor antagonist SR141716A has been reported to produce both anxiogenic (Arevalo et al., 2001) and anxiolytic (Haller et al., 2002) actions. However, the reduction of anxiety was not likely to be due to antagonism at CB_1 receptors, since it was observed also in cannabinoid CB_1 knockout mice (Haller et al., 2002). These opposing results have not helped to clarify the issue of whether endocannabinoids tonically enhance or reduce anxious behaviors. Furthermore, depending on the background strain used to inactivate the cannabinoid CB_1 receptor gene, either enhanced (Haller et al., 2002; Martin et al., 2002) or unaltered (Marsicano et al., 2002) anxiety have been reported in CB_1 knockout mice using the "elevated plus maze" or the "lit–dark box" tests. A very recent study, however, provided support to the hypothesis that endocannabinoids, and anandamide in particular, exert an anxiolytic tone in rodents, by showing that selective inhibitors of fatty acid amide hydrolase, while enhancing anandamide levels in the brain, also reduce anxiety in the "elevated zero maze" and the "isolation-induced ultrasonic vocalization" tests in rats, in a way blocked by SR141716A (Kathuria et al.,

2003). This important finding needs now to be further validated by assessing how fatty acid amide hydrolase knockout mice, which also contain significantly elevated anandamide brain levels (Cravatt et al., 2001), react in these tests of anxiety. Furthermore, the neural and neurochemical substrates of the possible anxiolytic role of the endocannabinoid system still represents an entirely open field of investigation.

6. Concluding remarks

We have described here data published in the literature pointing to involvement of the endocannabinoid system in neurological and psychiatric disorders, with particular emphasis on the possible role played in this context by the capability of cannabinoid CB_1 receptors to interfere, either directly, or more probably, indirectly, with the activity dopaminergic neurons. Furthermore, we have mentioned evidence suggesting that endocannabinoids intervene in neuropsychiatric dysfunctions also independently of their actions on dopamine signaling, such as, for example, anxiety and eating disorders. From the data described here and in our other review in which we have discussed the actions of the endocannabinoid system during neurodegeneration (van der Stelt et al., 2002), a general, although still quite speculative, scenario emerges where endocannabinoid signaling might be seen as an adaptive response to stimuli or conditions that pose a threat to the organism, and to the brain in particular. These conditions can be triggered by either inflammatory, mechanically and psychologically stressful stimuli, such as excitotoxicity or neurodegeneration, abstinence from food/liquid intake, drug abuse, excessive fear, etc. Via its general inhibitory actions, endocannabinoids are able to compensate, both at the neurochemical and behavioral level, for the abnormal neurotransmission caused by these conditions. For example, the endocannabinoid system may reduce glutamatergic transmission during excitotoxicity and neurodegeneration, modulate GABAergic transmission in anxiety disorders or modify dopaminergic signaling in addiction and schizophrenia. However, this endogenous ‘‘anti-stress’’ response may cause other effects, which can contribute to some of the symptoms of the disorders observed during their progression, as observed in animal models of Parkinson’s disease or during the relapse to drug abuse.

Several studies still need to be performed in order to substantiate or discard this hypothesis, and before the use of endocannabinoid-derived drugs (i.e., inhibitors of endocannabinoid biosynthesis, action or inactivation) (Di Marzo et al., 2000a) can be proposed as therapeutic remedies for the alleviation of the symptoms of psychiatric disorders. Furthermore, the possible role of vanilloid VR1 receptors that are targeted by at least two of the five endocannabinoids discovered so far and appear to be expressed to some extent in dopaminergic neurons (see Di Marzo et al., 2002 for a review), also needs to be investigated further. It is possible that anandamide or *N*-arachidonoyldopamine exert also vanilloid VR1 receptor-mediated neuromodulatory actions on dopamine signaling, either directly or indirectly via: (i) stimulation of glutamate release, as recently demonstrated for some neurons of the substantia nigra compacta (Marinelli et al., 2003), or (ii) stimulation of GABA release, a phenomenon postulated to explain the enhancement of paired-pulse inhibition in the CA1 region of the hippocampus (Al-Hayani et al., 2001; Huang et al., 2002). These studies might reveal an unprecedented complexity for endocannabinoid regulation of dopaminergic signaling and, hence, of neurological function and behavior under physiological and, particularly, pathological conditions.

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