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Role of the Endogenous Cannabinoid System as a Modulator of Dopamine Transmission: Implications for Parkinson's Disease and Schizophrenia

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The endogenous cannabinoid system is a new signaling system composed by the central (CB_1) and the peripheral (CB_2) receptors, and several lipid transmitters including anandamide and 2-arachidonylglycerol. This system is the target of natural cannabinoids, the psychoactive constituents of *Cannabis sativa* preparations (marijuana, hashish). Acute and chronic cannabis exposure has been associated with subjective feelings of pleasure and relaxation, but also to the onset of psychiatric syndromes, a decrease of the efficacy of neuroleptics and alterations in the extrapyramidal system regulation of motor activity. These actions points to a tight association of the cannabinoid system with the brain dopaminergic circuits involved in addiction, the clinical manifestation of positive symptoms of schizophrenia and Parkinson's disease. The present work discuss anatomical, biochemical and pharmacological evidences supporting a role for the endogenous cannabinoid system in the modulation of dopaminergic transmission. Cannabinoid CB_1 receptors are present in dopamine projecting brain areas. In primates and certain rat strains it is also located in dopamine cells of the A8, A9 and A10 mesencephalic cell groups, as well as in hypothalamic dopaminergic neurons controlling prolactin secretion. CB_1 receptors co-localize with dopamine D_1/D_2 receptors in dopamine projecting fields. Manipulation of dopaminergic transmission is able of altere the synthesis and release of anandamide as well as the expression of CB_1 receptors. Additionally, CB_1receptors can switch its transduction mechanism to oppose to the ongoing dopamine signaling. Acute blockade of CB_1 receptor potentiates the facilitatory role of dopamine D_2 receptor agonists on movement. CB_1 stimulation results in sensitization to the motor effects of indirect dopaminergic agonists. The dynamics of these changes indicate that the cannabinoid system is an activity-dependent modulator of dopaminergic transmission, an hypothesis relevant for the design of new therapeutic strategies for dopamine-related diseases such as the psychosis and Parkinson's disease.

INTRODUCTION

The endocannabinoid system in the brain is configured by the central cannabinoid receptor, CB_1 (Devane *et al.*, 1988), and the endogenous ligands anandamide (Devane *et al.*, 1992) and 2-arachidonyl glycerol (Mechoulam *et al.*, 1995; Stella *et al.*, 1997). Acute and chronic effects of *Cannabis sativa* derivatives on central nervous system

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functioning are mediated through the action of THC, its main psychoactive constituent (Gaoni and Mechoulam, 1964) on specific brain CB_1 cannabinoid receptors. The potential adverse effects of *Cannabis* consumption may include, among others, anxiety-like disorders (Halikas *et al.*, 1985; Rodríguez de Fonseca *et al.*, 1997), increased risk for the onset of psychotic syndromes (Andréasson *et al.*, 1987; Nuñez-Domínguez and Gurpegui-Fernández, 1997) and a decrease of the therapeutic effectiveness of neuroleptics (Knudsen and Vilmar, 1984). The psychopathological features associated to acute and chronic *Cannabis* exposure points to a tight connection between the endogenous dopamine transmission systems (dopamine- releasing and dopamine receptor-expressing neurons) and the endogenous cannabinoid system. Dopamine neurons, mainly those from the nigrostriatal and the mesocorticolimbic pathways, have been considered relevant for the process of reward and stress signals, drug addiction, and the positive symptoms of schizophrenia (Grace, 1991; Le Moal and Simon, 1991).

There is a growing literature confirming the above proposed contribution of the endogenous cannabinoid system in dopamine-related diseases such as addiction (Gardner and Vorel, 1998; Navarro *et al.*, 1998), stress (Rodríguez de Fonseca *et al.*, 1997), psychoses (Andréasson *et al.*, 1987; Knudsen and Vilmar, 1984; Nuñez-Domínguez and Gurpegui-Fernández, 1997) or extrapyramidal disorders such as Parkinson's disease or dystonias (Clifford, 1983; Glass *et al.*, 1997; Rodríguez de Fonseca *et al.*, 1994b and 1998). However, in order to understand that contribution to dopamine-related neuropsychiatric conditions we need to solve one of the striking challenges in the cannabinoid field: the explanation of the physiological role of a system densely present in dopamine-projecting brain areas, with a highly preserved neurobiological properties throughout the evolution, but with a low tonic activity as revealed by functional antagonism studies (Howlett, 1995; Gueudet *et al.*, 1995; Navarro *et al.*, 1997). In the present work we will briefly discuss the biochemical, anatomical and behavioral components of the interaction between dopamine and endocannabinoid systems. We will propose a model under which explore the potential relevance of these interactions for the understanding and treatment of neurodegenerative disorders such as Parkinson's disease, and psychiatric syndromes such as schizophrenia.

NEURONATOMICAL ANALYSIS OF THE PRESENCE OF CANNABINOID RECEPTORS IN BRAIN DOPAMINE CIRCUITS

Cannabinoid CB_1 receptors are distributed in the mammalian brain at a higher levels than any other known G-protein-coupled receptor (Herkenham *et al.*, 1990; Mailleux and Vanderhaeghen, 1992; Matsuda *et al.*, 1990 and 1993; Tsou *et al.*, 1998). They are expressed in areas of the central nervous system that contribute to the control of movement (caudate-putamen, globus pallidum, entopeduncular nucleus, substantia nigra and cerebellum), memory and cognition (hippocampal formation, cingulate cortex), processing of emotions and motivational responses (amygdalar complex, nucleus accumbens, olfactory cortex), pain perception (central gray matter, dorsal horn of spinal medulla), and neuroendocrine integration (paraventricular, arcuate, supraoptic and ventromedial hypothalamic nuclei). (Mailleux and Vanderhaeghen, 1992; Matsuda *et al.*, 1993). The analysis of this distribution reveals that the pharmacological profile described for CB_1 agonists (Dewey, 1986; Howlett, 1995) matches with the anatomical distribution of both CB_1 -agonist binding sites and CB_1 mRNA. Interestingly, cannabinoid receptor antagonist SR141716A (Rinaldi-Carmona *et al.*, 1994) has been used to confirm this specific neuroanatomical profile, and has revealed the existence of an endogenous cannabinoid tone in the hippocampus, substantia nigra and limbic sys-

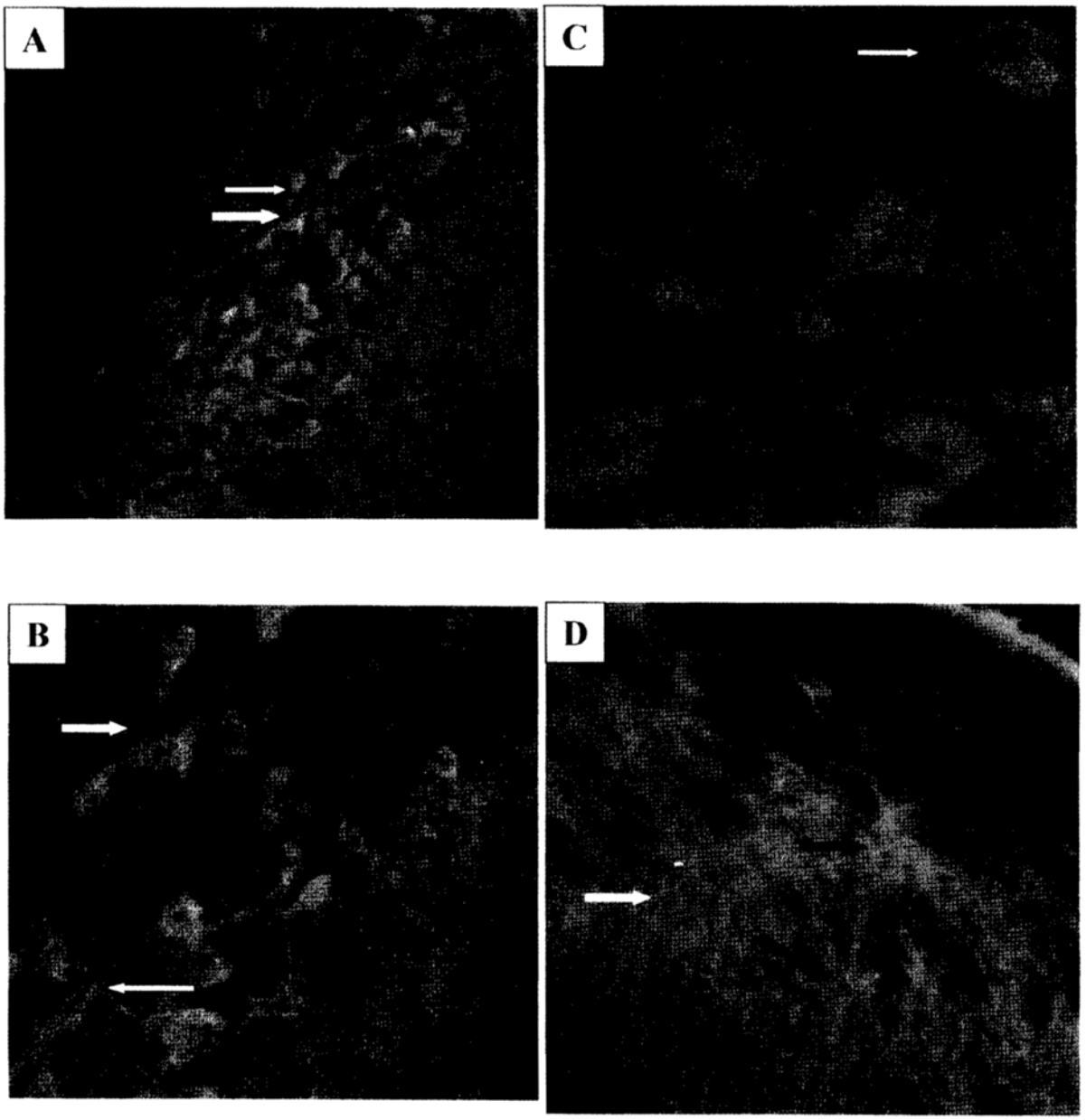

FIGURE 1 Neuroanatomical distribution of CB_1 receptors in the substantia nigra of the Wistar rat brain, as revealed by immunocytochemical double labelling and confocal microscopy. Red flourescence corresponds to tyrosine hydroxylase (TH) immunopossitive cells, whereas green flourescence reveals the location of cannabinoid CB_1 receptors. Double labelling appears as a yellow/orange flourescence. Details on the method have been previously described (Rodríguez de Fonseca *et al.*, 1999). **A**. Panoramical view of the substantia nigra pars compacta showing cells labelled only for TH (white arrow) or double-labelled for TH and CB_1 (yellow arrow). **B** and **C**. Higher magnification of the external border of the pars compacta, showing those cells and cells only labelled for CB_1 receptor (red arrow). **D**. Panoramical view of the substantia nigra showing TH-labelled dendrites from the pars compacta (white arrow) penetrating into the pars reticulata which expres CB_1-receptor immunopossitive terminals (white arrow) (See Color Plate I at the back of this issue)

tem which also correlates with the neuroanatomical distribution of CB_1 receptors (Gessa *et al.*, 1997; Gueudet *et al.*, 1995; Navarro *et al.*, 1997; Rodríguez de Fonseca *et al.*, 1997).

Although cannabinoid CB_1 receptors were formerly described in specific brain locations related to dopamine circuits, such as the basal ganglia, the extended amygdala and the limbic cortices, they seemed to be absent in brain dopaminergic neurons (Herkenham *et al.*, 1990; Matsuda *et al.*, 1993). However recent studies (Ong and Mackie, 1999; Rodríguez deFonseca et al, unpublished, see Figure 1) have revealed strain and species-specific differences in the distribution of CB_1 receptors in brain dopamine cells. We have found that in the Wistar rat, as opposed to the deeply studied Sprague-Dawley strain (Herkenham *et al.*, 1991; Matsuda *et al.*, 1993; Tsou *et al.*, 1998), dopaminergic cells express low to moderate amount of CB_1 receptors. Similar findings were reported in the primate brain, (Ong and Mackie, 1999), supporting the possibility of a direct action of CB_1 receptor agonists on dopamine neurons, as suggested in early electrophysiological studies using pharmacological antagonists of the CB_1 receptor (French *et al.*, 1997; Guedet *et al.*, 1995)

It is generally accepted is that CB_1 receptors co-localize with dopamine receptors in neurons of dopamine-projecting fields such as the basal ganglia and limbic cortex (Herkenham *et al.*, 1991; Mailleux and Vanderhaeghen, 1993). Both kind of receptors are mostly found in GABAergic projecting neurons, (although glutamatergic neurons expressing CB_1 receptors have been reported, including those from the cortex and the subthalamic nuclei, Rodríguez de Fonseca *et al.* 1998; Sañudo-Peña and Walker, 1997). In the basal ganglia circuitry, GABAergic medium-spiny striatal neurons that express CB_1 receptors, receive afferents from dopamine neurons of the substantia nigra pars compacta and show co-expression of dopamine D_1, D_2 and D_3 receptors (Surmeier *et al.*, 1996). Their axon terminals innervating the globus-pallidum, substantia nigra pars reticulata and subthalamic nucleus contain high amounts of CB_1 receptors (Herkenham *et al.*, 1991). Figure 2 illustrates a typical image of functional cannabinoid CB_1 receptors in the mesencephalom of the rat, mapped by CB_1 agonist-stimulated GTPγS incorporation (Sim *et al.*, 1996). The dense activation of the substantia nigra, mainly the pars reticulata, indicates the potential relevance of the endogenous cannabinoid system in basal ganglia functioning.

FUNCTIONAL ASPECTS OF CANNABINOID-DOPAMINE INTERACTIONS

In the brain areas described above, relevant for most neuropsychiatric diseases, CB_1stimulation might either directly modulate the activity of dopaminergic neurons or interfere with the transduction of dopamine signal at postsynaptic dopamine receptors co-localized with CB_1. The presence of CB_1 receptors in dopamine cells allow a direct regulation of dopaminergic activity (i.e. spontaneous and evoked firing, synthesis and release of dopamine, etc...) by the endogenous cannabinoid system.

Several experimental approaches have shown both possibilities (for review see Gardner and Vorel, 1998; Rodríguez de Fonseca et al, 1998). Interaction between dopamine receptors and CB_1 receptors has a neurobiological support in the similar structure of both receptorial systems. These receptors belong to the family of G-protein coupled receptors for neurotransmitters (Matsuda *et al.*, 1990; Howlett, 1995). Both types of receptors are coupled to the same transduction systems, including the control of cAMP synthesis, and the regulation of $Ca^{2+}$and K^+ channels (Hampson *et al.*, 1995; Howlett, 1995; Mackie and Hille, 1992).

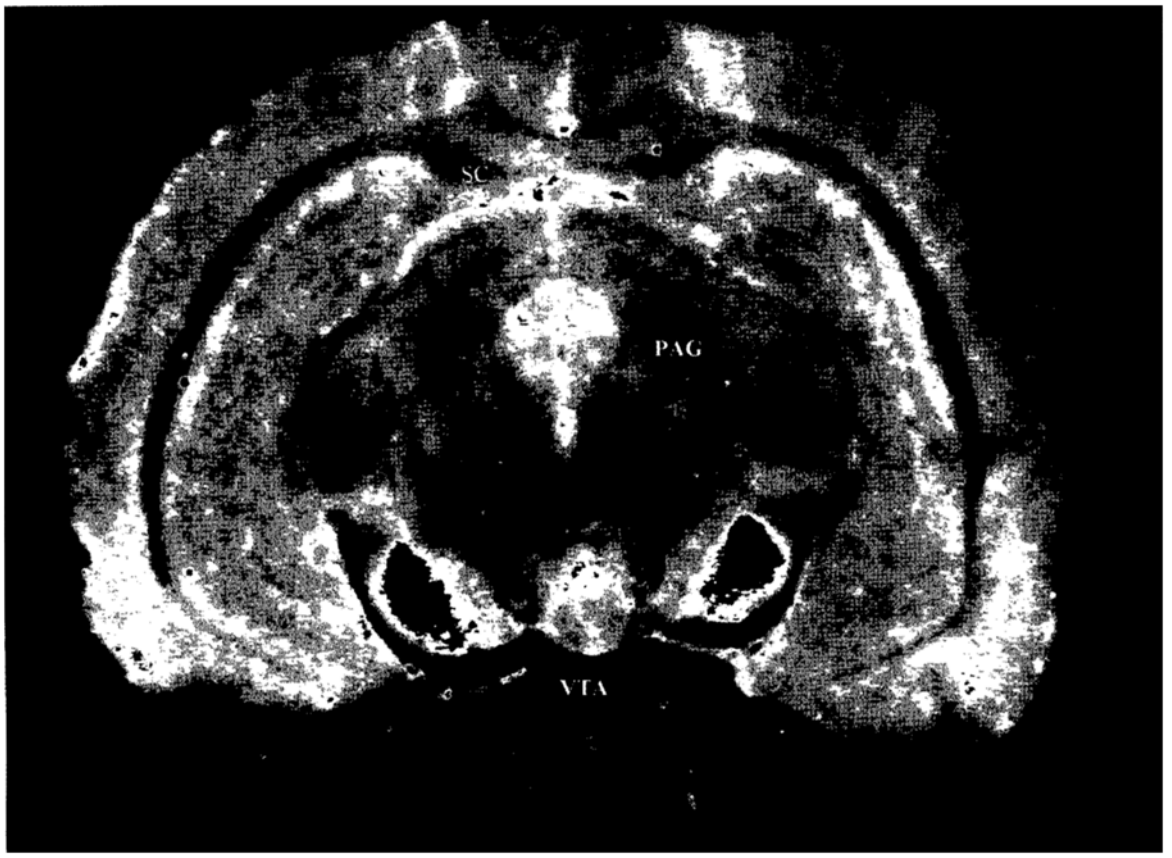

FIGURE 2 Coronal autoradiography showing the stimulatory effects of the cannabinoid CB_1 receptor agonist WIN 55,212–2 on GTPγ-S incorportion in the mesencephalon of the rat brain. WIN 55,212–2 (5 μM) produced a marked incorporation of GTPγ-S in the substantia nigra of the rat brain, which appear heavy labelled (**SN**, red color). Other areas specifically labelled were the ventral tegmental area (**VTA**), the periaqueductal graymatter (**PAG**) and the superficial layer of the superior colliculus (**SC**) (See Color Plate II at the back of this issue)

Dopamine-cannabinoid interactions in the nigrostriatal system

At the level of the different dopamine circuits, the role of the endogenous cannabinoid system seemed to be different. In the *nigrostriatal* pathway most of the effects observed after CB_1 receptor stimulation point to an indirect regulation of dopaminergic activity, through the action of CB1 receptor expressing GABAergic neurons of the outflow nuclei of basal ganglia (Maneuf *et al.*, 1996, Navarro *et al.*, 1993b; Pertwee and Greentree, 1988). Striatal dopamine turnover was not found to be affected after acute THC exposure neither in Sprague-Dawley (Rodríguez de Fonseca *et al.*, 1992) nor in Wistar rats (Navarro *et al.*, 1993b). However, acute systemic administration of cannabinoid receptor agonists were found to induce a small increase in the spontaneous activity of nigrostriatal dopaminergic neurons measured by either extracellular recordings (French *et al.*, 1997) or by in vivo voltammetry in the striatum (Ng Cheong *et al.*, 1988). The direct or indirect nature of these effects remains to be conclusively determined. However, a recent

study has partially clarified the links between the endogenous cannabinoid system and dopamine transmission. (Giuffrida *et al.*, 1999). In this study we have demonstrated by *in vivo* microdialysis that the extracellular levels of anandamide in the dorsal striatum are greatly increased after the activation of dopamine D_2family of receptors (i.e. after quinpirole infused by reverse dialysis), but not after stimulation of dopamine D_1 receptors (i.e. after infusion with the D_1 agonist SKF 38393). The D_2 -receptor evoked anandamide release may serve to limit the extent of behavioral activation induced by dopamine in the striatum. These findings are in agreement with other reports showing an increased behavioral response to the indirect dopamine receptor agonist amphetamine in animals chronically treated with the cannabinoid receptor agonist THC and displaying down-regulated CB_1 receptors (Gorriti *et al.*, 1999; Rodríguez de Fonseca *et al.*, 1994a). As shown in figure 3, the pretreatment with the CB_1 antagonist SR141716A potentiates the stimulation of motor behavior elicited by systemic administration of quinpirole. This effect was not observed when the facilitatory effect on movement was induced by a D_1 agonist (Figure 4). Other laboratories have described pharmacological interactions between dopamine D_2 receptor and cannabinoid CB_1 receptors which support this model. Thus, the group of J.M. Walker has shown that regional administration of D_2 family of agonists opposes the behavioral responses to the injection of CB_1agonists (Sañudo-Peña *et al.*, 1996; Sañudo-Peña and Walker, 1998).

Although the triggering of anandamide release seems to be dependent on D_2 receptor stimulation, the dopamine D_1 receptors plays also an important role in these cannabinoid-dopamine interaction. Thus, a previous report (Mailleux and Vanderhaeghen, 1993) demonstrated that the chronic blockade of D_1 receptors, which induce a compensatory hyperactivity in nigrostriatal dopaminergic cells, dramatically upregulated the expression of the CB_1 receptor mRNA in the dorsal striatum. Additional behavioural studies showed that CB_1 agonists blocked rotational behaviour induced by dopamine D_1 receptor agonists (Anderson *et al.*, 1995) whereas repeated stimulation of dopamine D_1 receptors (Rodriguez de Fonseca *et al.*, 1994) resulted in a potentiation of cannabinoid agonist-induced catalepsy and akinesia. These data suggest the existence of differences in the interactions between dopaminergic and cannabinoid systems regarding the different receptors involved. A current working hypothesis on the interaction between cannabinoid and dopamine receptors in the striatum is depicted in figure 5.

Whether these pharmacological effects are selective for the dorsal striatum or appear in other brain areas remains to be determined. Regional differences on the role of CB_1 receptors in controlling basal ganglia activity at mesencephalic sites have been proposed because of the dense presence of cannabinoid receptors in the substantia nigra (Glass *et al.*, 1997a). Thus, it has been found that cannabinoids activate substantia nigra pars reticulata neurons, probably by inhibiting GABA release from striatonigral projections through the stimulation of presynaptic CB_1 receptors (Tersigni and Rosemberg, 1996). This blockade of GABA release may be responsible for the *in vivo* observed increased activity of nigrostriatal dopamine neurons after acute cannabinoid exposure since they are under the influence of the striatonigral pathway (French *et al.*, 1997; Gueudet *et al.*, 1995; Ng Cheong *et al.*, 1988). An additional indirect source of regulatory inputs to the substantia nigra comes from the striatum through the subthalamic nucleus, whose activity may be modulated by CB_1stimulation (Sañudo-Peña and Walker, 1997). On the other hand, cannabinoid receptors in the striatum seem to be negatively coupled to K^+-stimulated (Navarro *et al.*, 1993) or electrically-evoked (Cadogan *et al.*, 1997) dopamine release, as revealed by *in vitro* studies. A balance between the activity of converging influences to the substantia nigra and local regulatory activi-

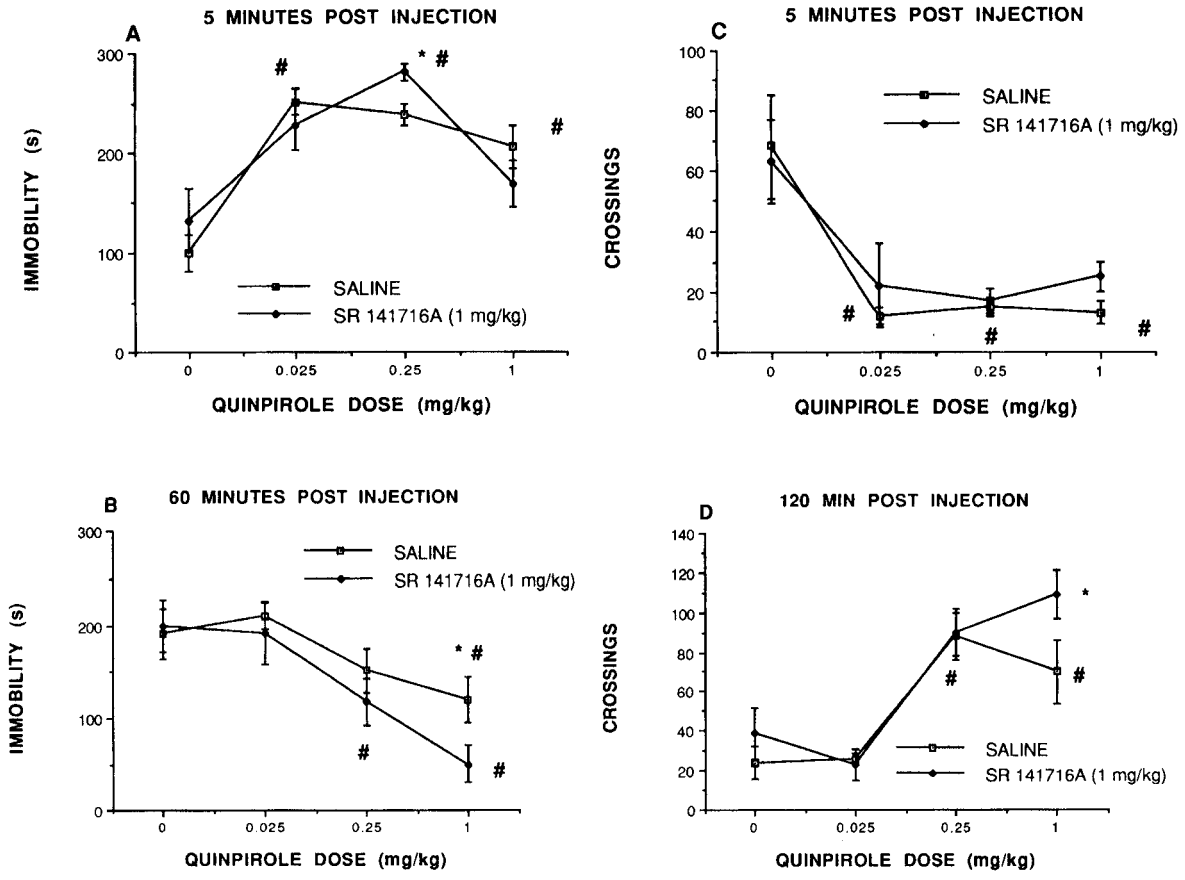

FIGURE 3 Effects of pretreatment with the cannabinoid CB_1 receptor antagonist SR141716A (1 mg/kg, i.p. 30 min. prior to second drug administration) on the acute effects of the dopamine D_2 receptor agonist quinpirole (0.025, 0.25 and 1 mg/kg, s.c.) on motor activity in male Wistar rats. Data were collected at different times after the injection of quinpirole (5, 60 or 120 min) to reveal the characteristic time and dose-dependent biphasic components of dopamine D_2 receptor agonists on motor behavior: an initial early inhibitory component derived of the stimulation of presynaptic D2 receptors, and a late stimulatory component derived of the activation of postsynaptic receptors. Data were expressed as total time spent in absolute quietness (immobility, upper pannels) or the number of crossings scored in a standard open field test (crossings, lower panels). Blockade of CB_1 receptors with SR141716A potentiates quinpirole-induced alterations on behavior, supporting for a role of the endogenous cannabinoid system in regulating dopamine facilitation of motor behavior. * $P < 0.05$ vehicle *versus* SR141716A-treated animals, # $P < 0.05$ *versus* vehicle-treated (0 dose) animals of the same pretreatment, Newman-Keuls

ties will then establish the nature of the actions of CB_1 agonists on the dopamine release in the striatum. Pharmacological manipulations of the acute sensitivity to cannabinoids by concurrent administration of GABAA and GABAB receptor acting drugs (Pertwee and Greentree, 1988; Romero *et al.*, 1995) support the involvement of GABA neurons in the mediation of cannabinoid effects. This contribution can also be deduced from the finding of CB_1 receptor agonist-induced changes in striatal dopamine receptors (Navarro *et al.*, 1993b; Rodríguez de Fonseca *et al.*, 1992) or by the profound alterations in neuropeptide gene expression found in striatal GABAergic cells of CB_1 receptor-knockout mice (Steiner *et al.*, 1999).

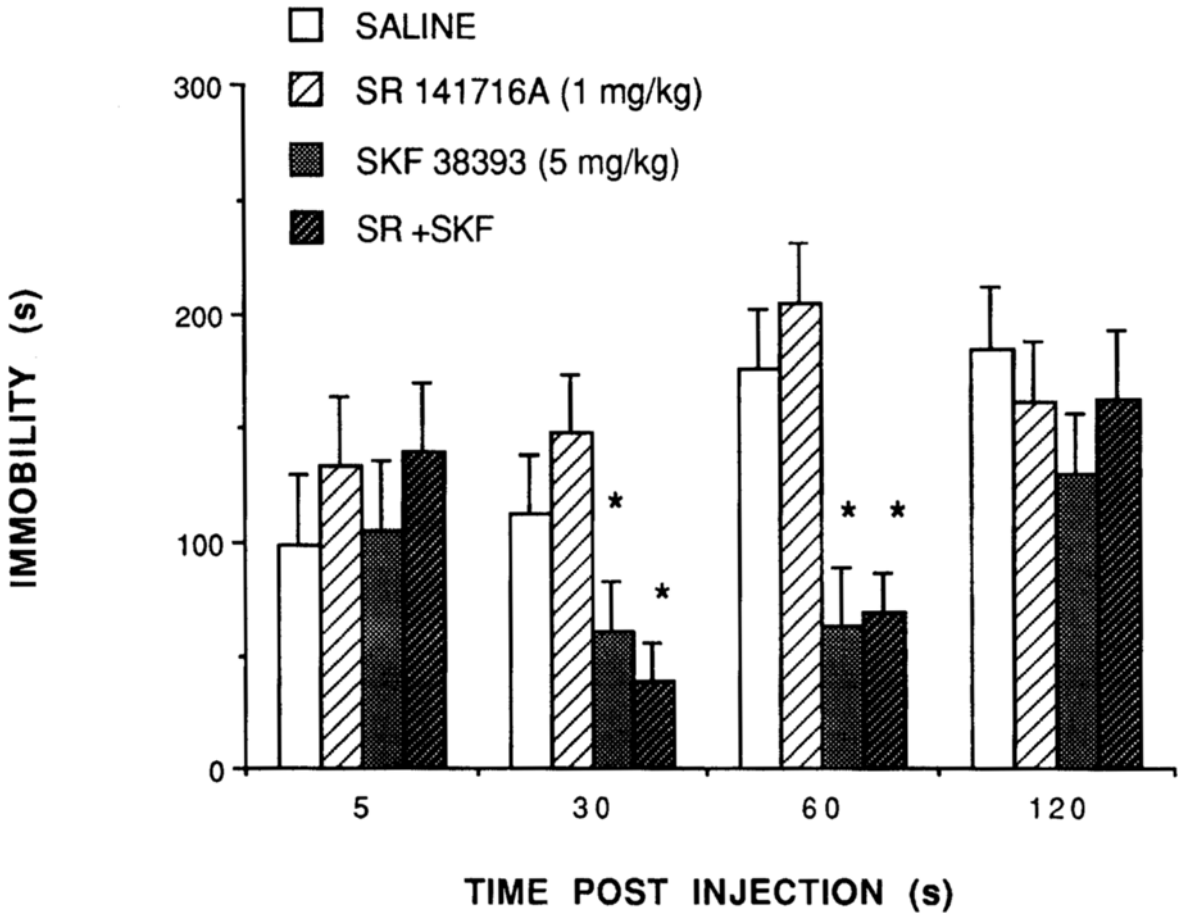

FIGURE 4 Lack of effects of pretreatment with the cannabinoid CB_1 receptor antagonist SR141716A (1 mg/kg, i.p. 30 min. prior to second drug administration) on the acute facilitatory effects of the dopamine D_1 receptor agonist SKF 38393 (5 mg/kg, s.c.) on motor activity in male Wistar rats. Data were expressed as total time spent in absolute quietness (immobility, upper pannels). They were collected at different times (5, 30, 60 or 120 min) after the injection of the D1 agonist.* $P < 0.05$ *versus* saline-treated animals, Newman-Keuls

Dopamine-cannabinoid interaction in the mesocorticolimbic circuits

The *mesocorticolimbic* dopaminergic system has been found to be more sensitive than the nigrostriatal patway to the acute administration of cannabinoids (Gardner and Vorel, 1998). First descriptions of THC actions in the brain pointed to an stimulation of mesocorticolimbic dopaminergic activity (Chen *et al.*, 1990;). Direct extracellular recordings showed that systemic administration of CB1 agonists increased the activity of ventral tegmental area dopaminergic neurons (French *et al.*, 1997), associated to an increased dopamine release in the mesolimbic targets (Chen *et al.*, 1990; Gardner *et al.*, 1988; Gardner and Vorel 1998). However, whether this effect is produced by direct stimulation of CB1 receptors present in dopaminergic cells or by transynaptic stimulation remains to be conclusively determined. In this respect, further research is required to establish a role for

dopamine D_2 and D_1 receptors on the response of mesocorticolimbic dopaminergic cells to acute cannabinoid exposure. On the other hand, blockade of CB1 receptors with SR 141716A does not affect ventral tegmental area activity (Gueudet *et al.*, 1995), whereas μ-opioid receptor antagonists (naloxone, naloxonazine) block the increased dopamine release induced by CB1 agonists administration, acting probably both at the ventral tegmental area neurons (Tanda *et al.*, 1997) and at their projecting terminals in the nucleus accumbens (Chen *et al.*, 1990; Gardner and Vorel, 1998). An interesting additional hypothesis is the possible glucocorticoid dependence of CB1 agonists-induced mesolimbic activation. Cannabinoids are chemical stressors which activate the pituitary adrenal axis by stimulating ACTH release (Martín-Calderón *et al.*, 1998). They also induce anxiety-like responses (Rodríguez de Fonseca *et al.*, 1997). Acute stress is associated with a rapid activation of mesolimbic circuitry that can be mediated by glucocorticoid receptors present in mesolimbic dopaminergic neurons (Piazza *et al.*, 1996). Whether cannabinoid-induced activation of mesolimbic activity depends on the activation of pituitary-adrenal axis remains to be conclusively determined, but its demonstration may help support clinical observations on the role of *Cannabis* comsumption as a vulnerability factor for the onset of psychosis or drug addiction (Andreasson *et al.*, 1987; Rodríguez de Fonseca *et al.*, 1997).

CANNABINOID-DOPAMINE INTERACTIONS AND DOPAMINE-RELATED DISEASES: WHAT IS NEXT?

As described above, endogenous cannabinoids are local mediators released to regulate information processing within the main relays of the basal ganglia nuclei. The suggested constitutive activity of the CB1 receptor and its potential bidirectional coupling to the adenylate cyclase suggest that this regulatory function affects processes of opposed nature within the striatum, indicating a potential role for this system as an homeostatic set-point mechanism (Glass and Felder, 1997b; Maneuf and Brotchie, 1997; Rodríguez de Fonseca et al., 1998). The induction of a functional blockade of neurotransmitter uptake processes derived from CB_1 stimulation can affect to neurotransmitters of opposed nature, such as glutamate and GABA, supporting again this buffering role for the endogenous cannabinoid signalling within striatum (Maneuf *et al.*, 1996). Much work is needed in order to identify the potential regional variabilities in these mechanisms, as well as the pathological conditions on which a clear contribution of the endogenous cannabinoid system may enhance our knowledge of neurological disorders.

The research findings discussed in this manuscript suggest that the endogenous cannabinoid system may serve as a target for the development of new strategies for the treatment of dopamine-related diseases, such as motor syndromes. Among those movement disorders, Parkinson's Disease and neuroleptic-induced dyskinesias and dystonias are firmly clinical entities that may be benefitiated from therapy based on the endogenous cannabinoid system, together with Gilles de la Tourette syndrome and Huntington's chorea (Rodríguez de Fonseca *et al.*, 1998). As a potential practical utility of the model we can propose cannabinoid agonist for reducing unwanted effects of L-DOPA or dopaminergic agonists in Parkinson's disease or neuroleptic associated tardive dyskinesias, as well as a cannabinoid antagonist for reducing the effective dose of L-DOPA or dopamine agonists needed to alleviate akinesia (Maneuf *et al.* 1997; Rodríguez de Fonseca *et al.*, 1998).

Besides motor disorders several neuropsychiatric conditions could also benefit from the availability of new compounds acting at the cannabinoid receptors, such as new and selective antagonists. That goal can also be addressed by designing drugs which may interfere with new

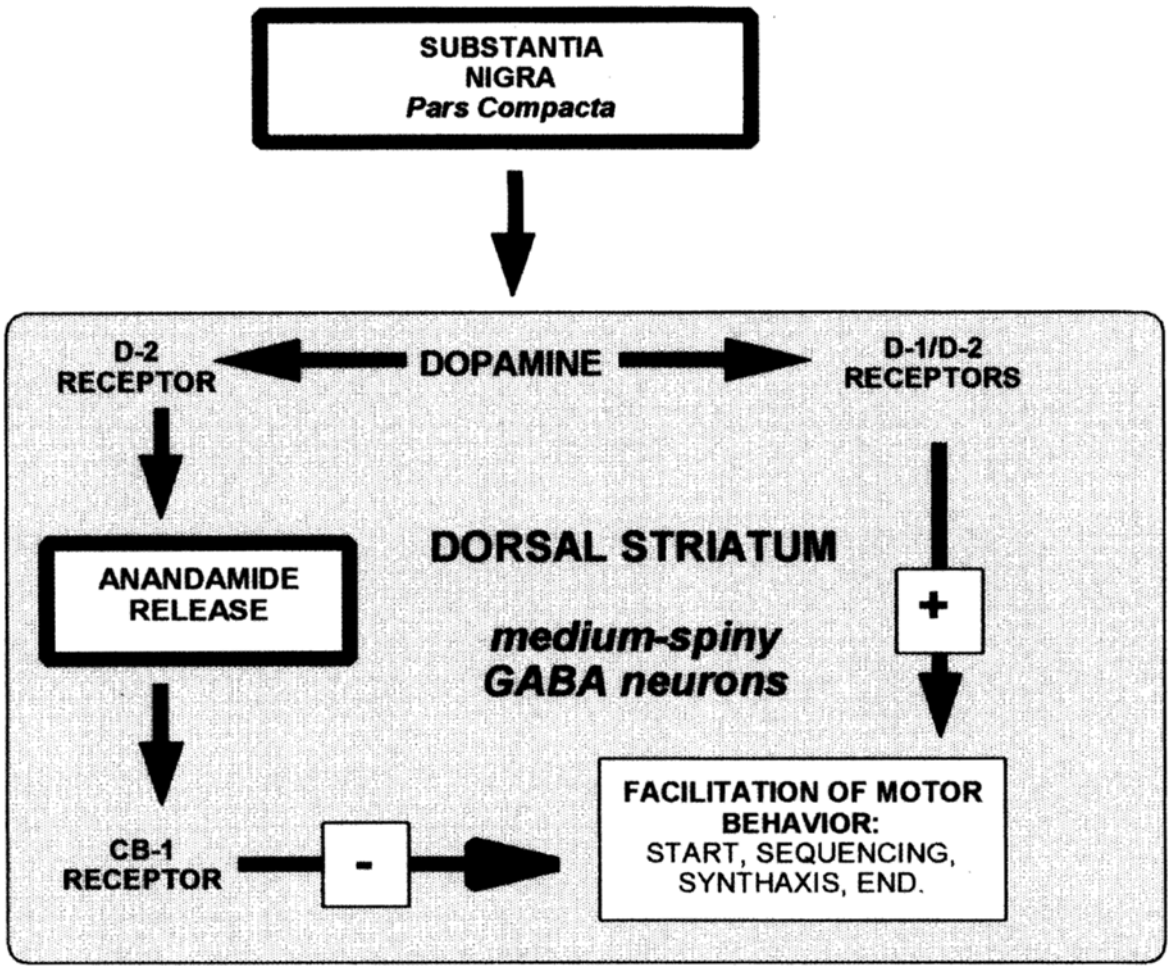

FIGURE 5 A proposed model for the interactions between dopaminergic transmission and the endogenous cannabinoid system in the dorsal striatum. Dopamine released from axon terminals of substantia nigra pars compacta neurons facilitates motor behavior through the activation of both dopamine D_2 and D_1 receptors located in medium-spiny GABAergic neurons of the dorsal striatum. Simultaneously, activation of dopamine D_2 receptors triggers the release of anandamide from a plasmalemma precursor, which in turn limited the activatory effects of dopamine by stimulating CB_1 receptors. Although depicted as a postsynaptic mechanism, anandamide could be originated through the activation of presynaptic D_2 receptors, and eventually may act at presynaptic CB_1 receptors

molecular mechanisms involved in cannabinoid transmission, such as the recently described anandamide transporter (Beltramo *et al.*, 1997). This latest target has open multiple possibilities for the treatment of dopamine-related diseases. A cardinal example could be the acute symptoms of schizophrenia, which are currently attributed, among other mechanisms, to an hyperdopaminergic state. A depicted in figure 5, anandamide can act as a local mediator regulating dopamine activity. If anandamide release is also triggered in cortical areas upon dopamine D_2 receptor activation, as decribed in the dorsal striatum (Giuffrida *et al.*, 1999), an enhancement of anandamide levels through the pharmacological blockade of anandamide uptake or hydroly-

sis may act as a neuroleptic through its inhibitory action on dopamine receptor-evoked responses. In support of this hypothesis, preliminary reports have described the presence of elevated levels of anandamide in the cerebrospinal fluid of schizophrenia patients (Leweke *et al.*, 1999). An additional advantage of such therapeutic approach derives from the fact that anandamide is a partial agonist: the uptake blocker-induced rise in synaptic anandamide may not produce the unwanted side effects, including catalepsy, acute stress-like reactions or the fort receptor desensitization that characterize the action of full CB1 receptor agonists (Dewey, 1986; Howlett, 1995).

The buffering effects of cannabinoids on synaptic transmission processes points to a neuroprotectant role for drugs aimed to potentiate cannabinoid transmission. Although glucocorticoid-dependent neuronal loss in the hippocampus has been found in rats after chronic treatment with THC (Landfield et al, 1988) neuroprotective actions derived of cannabinoid CB_1 receptor stimulation have been described both *in vivo* and *in vitro* (Nagayama et al., 1999). These findings indicate that *a rational* use of drugs aimed to the cannabinoid CB_1receptor may protect neurons from different types of injuries derived of overstimulation of glutamate or dopamine release in central synapses.

Acknowledgements

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References

Anderson LA, Anderson JJ, Chase TN and Walters JR (1995) The cannabinoid agonist WIN 55,212–2 and CP 55,940 attenuate rotational behaviour induced by a dopamine D1 but not a D2 agonist in rats with unilateral lesions of the nigrostriatal pathway. *Brain Res.*, **691**, 106–114.

Andreàsson S, Allebeck P, Engström A, Rydberg V (1987) Cannabis and Schizophrenia: a longitudinal study of Swedish conscripts. *Lancet*, **ii**, 1483- 1486.

Beltramo M, Stella N, Calignano A, Lin SY, Makriyannis A and Piomelli D (1997) Functional role of high-affinity anandamide transport as revealed by selective inhibition. *Science*, **277**, 1094–1097.

Cadogan AK, Alexander SPH, Boyd EA and Kendall DA (1997) Influence of cannabinoids on electrically evoked dopamine release and cAMP generation in the rat striatum. *J. Neurochem.*, **69**, 1131–1137.

Chen J, Paredes W, Li J, Smith D, Lowinson J and Gardner EL (1990) Delta-9-tetrahydrocannabinol produces naloxone-blockable enhancement of presynaptic basal dopamine efflux in nucleus accumbens of conscious, freely-moving rats as measured by intracerebral microdialysis. *Psychopharmacol.*, **102**, 156–162.

Clifford DB (1983) Tetrahydrocannabinol for tremor in multiple sclerosis. *Ann. Neurol.*, **13**, 669–671.

Devane WA, Dysarz FA III, Johnson MR, Melvin LS and Howlett AC (1988) Determination and characterization of a cannabinoid receptor in rat brain. *Mol. Pharmacol.*, **34**, 605–613.

Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A and Mechoulam R (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Nature*, **258**, 1946–1949.

Dewey WL (1986) Cannabinoid Pharmacology. *Pharmacol. Rev.*, **38**, 151–178.

French ED, Dillon K and Wu x (1997) Cannabinoids excite dopamine neurons in the ventral tegmentum and substantia nigra. *NeuroReport*, **8**, 649–652.

Gaoni Y and Mechoulam R (1964) Isolation, structure and partial synthesis on an active constituent of hashish. *J Am. Chem. Soc.*, **86**, 1646–1654.

Gardner EL, Paredes W, Smith D, Donner A, Milling C, Cohen D and Morrison D (1988) Facilitation of brain stimulation reward by δ^9-tetrahydrocannabinol. *Psychopharmacol.*, **96**, 142–144.

Gardner EL and Vorel, RH (1998) Cannabinoid transmission and reward-related events. *Neurobiology of Disease.* **5**, 502–533.

Gessa, GL, Melis, M, Muntoni AL, Diana M (1998) Cannabinoids activate mesolimbic dopamine neurons by an action on cannabinoid CB1 receptors. *Eur. J. Pharmacol.* **341**, 39–44.

Giuffrida, A., Parsons, L.H., Kehrr, A., Rodríguez de Fonseca, F., Navarro, M., Piomelli, D. (1999) Dopamine activation of endogenous cannabinoid signalling in dorsal striatum. *Nature Neuroscience* **2**, 358–363.

Glass M, Brotchie JM and MAneuf YP (1997a) Modulation of neurotransmission by cannabinoids in the basal ganglia. *Eur. J. Neurosci.*, **9**, 199–203.

Glass M and Felder C (1997b) Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors augments cAMP accumulation in striatal neurons: evidence for a Gs linkage to the CB1 receptor. *J. Neurosci.*, **17**, 5327–5333.

Gorriti, M.A., Rodriguez de Fonseca, F.A., Navarro, M., Palomo, M. (1999) Chronic treatment with (-)-delta-9-tet-

rahydrocannabinol results in sensitization to the psychomotor effects of amphetamine. *Eur. J. Pharmacol.* **365**, 133–142.

Grace AA (1991) Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: a hypothesis for the etiology of schizophrenia. *Neuroscience*, **41**, 1–24.

Gueudet C, Santucci V, Rinaldi-Carmona M, Soubrié P and Le Fur G (1995) The CB_1 cannabinoid receptor antagonist SR 141716A affects A9 dopamine neuronal activity in the rat. *NeuroReport*, **6**, 1293–1297.

Halikas JA, Weller RA, Mouse CL and Hoffman RA (1985) A longitudinal study of marijuana effects. *Int. J. Addiction*, **20**, 701–711.

Hampson RE, Evans GJO, Mu J, Zhuang S, King VC, Childers SR and Deadwyler SA (1995) Role of cyclic AMP dependent protein kinase in cannabinoid receptor modulation of potassium "A-current" in cultured hippocampal neurons. *Life Sci.*, **56**, 2081–2087.

Herkenham M, Lynn AB, de Costa BR and Richfield EK (1991) Neuronal localization of cannabinoid receptors in the basal ganglia of the rat. *Brain Res*, **547**, 267–274.

Herkenham M, Lynn AB, Little MD, Johnson MR, Melvin LS, de Costa BR and Rice (1990) Cannabinoid receptor localization in brain. *Proc. Natl. Acad. Sci. USA.*, **87**, 1932–1936.

Howlett AC (1995) Pharmacology of cannabinoid receptors. *Ann. Rev. Pharmacol. Toxicol.* **35**, 607–634.

Knudsen P and Vilmar P (1984) Cannabis and neuroleptics agents in schizophrenia. *Acta Psychiatr. Scand.*, **69**, 162–174.

Landfield PW, Cadwallader LB and Vinsant S (1988) Quantitative changes in hippocampal structure following chronic exposure to Δ^9-tetrahydrocannabinol: possible mediation by glucocorticoid systems. *Brain. Res.*, **443**, 47–62.

Le Moal M and Simon H (1991) Mesocorticolimbic dopaminergic network: functional and regulatory roles. *Physiol. Rev.*, **71**, 155–234.

Leweke, FM, Giuffrida, A, Wurster, U, Emrich, HM and Piomelli, D (1999) Elevated endogenous cannabinoids in schizophrenia. *Neuro Report* **10**, 1665–1669.

Mackie K, and Hille B (1992) Cannabinoids inhibit N-type calcium channels in neuroblastoma-glioma cells. *Proc. Natl. Acad. Sci. USA*, **89**, 3825–3829.

Mailleux P and Vanderhaeghen JJ (1992) Distribution of neuronal cannabinoid receptor in the adult rat brain: a comparative receptor binding radioautoagraphy and in situ hybridization histochemistry. *Neuroscience*, **48**, 655–688.

Mailleux P and Vanderhaeghen JJ (1993) Dopaminergic regulation of cannabinoid receptor mRNA levels in the rat caudate-putamen: An in situ hybridization study. *J. Neurochem.*, **61**, 1705–1712.

Maneuf YP and Brotchie JM (1997) Paradoxical action of the cannabinod WIN 55,212–2 in stimulated and basal cyclic AMP accumulation in rat globus pallidus slices. *Br. J. Pharmacol.*, **120**, 1397–1398.

Maneuf YP, Crossman AR and Brotchie JM (1996) Modulation of GABAergic transmission in the globus pallidus by the synthetic canabinoid WIN 55,212–2. *Synapse*, **22**, 383–385.

Maneuf YP, Crossman AR and Brotchie JM (1997) The cannabinoid receptor agonist WIN 55,212–2 reduces D2, but not D1, dopamine receptor-mediated alleviation of akinesia in the reserpine-treated rat model of Parkinson's disease. *Exp. Neurol.*, **148**, 265–270.

Martín-Calderón JL, Muñoz RM, Villanúa, del Arco I, Moren JL, Rodríguez de Fonseca F and Navarro M (1998) Characterization of the acute endocrine actions of HU-210, a potent synthetic cannabinoid in rats. *Eur. J. Pharmacol.* **344**, 77–86.

Matsuda LA, Bonner TI and Lolait SJ (1993) Localization of Cannabinoid Receptor mRNA in rat brain. *J. Comp. Neurol.*, **327**, 535–550.

Matsuda LA, Lolait SJ, Brownstein MJ, Young AC and Bonner TI (1990) Structure of a brain cannabinoid receptor and functional expression of the cloned cDNA. *Nature*, **346**, 561–564.

Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, Gopher A, Almog S, Martin BR, Comton DR, Pertwee RG, Griffin G, Bayewitch M, Barg J and Vogel Z (1995) Identification of an endogenous 2-monoglyceride present in canine gut that binds to cannabinoid receptor. *Biochem. Pharmacol.*, **50**, 83–90.

Nagayama, T., Sinor, A.D., Simon, R.P., Cheen, J., Graham, S.H., Jin, K., Greenberg, D.A. (1999) Cannabinoids and neuroprotection in global and focal cerebral ischemia and in neuronal cultures. *J. Neurosci.* **19**, 2987–95.

Navarro, M, Cowen, J, Carrera, MRA, del Arco, I., Villanúa, MA, Martin Y, Roberts, AJ, Koob, GF and Rodríguez de Fonseca, F (1998) CB1 cannabinoid receptor antagonist-induced opiate withdrawal in morphine-dependent rats. *NeuroReport* **9**, 3397–3402.

Navarro M, Fernandez-Ruiz JJ, de Miguel R, Hernandez ML, Cebeira M and Ramos JA (1993) Motor disturbances induced by an acute dose of delta-9-tetrahydrocannabinol: possible involvement of nigrostriatal dopaminergic alterations. *Pharmacol. Biochem. Behav.*, **45**, 291–298.

Navarro M, Hernández E, Muñoz RM, Del Arco I, Villanúa MA, Carrera MRA and Rodríguez de Fonseca F (1997) Acute administration of the CB_1 receptor antagonist SR 141716A induces anxiety-like responses in the rat. *NeuroReport*, **8**, 491–496.

Ng Cheong Ton JM, Gerhardt SA, Freidman M, Etgen A, Rose GM, Sharlegg NS and Gardner EL (1988) The effect of delta-9-tetrahydrocannabinol on potassium-evoked release of dopamine in the rat caudate nucleus: an in vivo electrochemical and in vivo microdialysis study. *Brain Res.*, **451**, 59–68.

Nuñez-Domínguez LA and Gurpegui-Fernández de Legaria M (1997) Cannabis psychosis: a five year follow-up study. *International Meeting on Interactive Monoaminergic Brain Disorders*. October 8–12, 1997, P3.5, pp 76.

Ong, W.Y. and Mackie, K. (1999) A light and electron microscopic study of the CB1 cannabinoid receptor in primate brain. *Neuroscience* **92**, 1177–1191.

Pertwee RG and Greentree SG (1988) Delta-9-tetrahydrocannabinol-induced catalepsy in mice is enhanced by pretreatment with flurazepam or chlordiazepoxide. *Neuropharmacol.*, **27**, 485–491.

Piazza PV and Le Moal M (1996b) Pathophysiological basis of vulnerability to drug abuse: role of an interaction between stress, glucocorticoids and dopaminergic neurons. *Ann. Rev. Pharmacol. Toxicol.*, **36**, 359–378.

Rinaldi-Carmona M, Barth F, Héaulme M, Shire D, Calandra B, Congy C, Martinez S, Maruani J, Néliat G, Caput D, Ferrara P, Soubrié, Breliére JC and Le Fur G (1994) SR

141716A a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Lett.*, **350**, 240–244.

Rodríguez de Fonseca F, Carrera MRA, Navarro M, Koob GF and Weiss F (1997) Activation of corticotropin-releasing factor in the limbic system during cannabinoid withdrawal. *Science*, **276**, 2050–2054.

Rodríguez de Fonseca F, Fernandez-Ruiz JJ, Murphy LL, Cebeira M, Steger RW, Bartke A and Ramos A (1992) Acute effects of δ^9-tetrahydrocannabinol on dopaminergic activity in several rat brain areas. *Pharmacol. Biochem. Behav.* **42**, 269–275.

Rodríguez de Fonseca F, Gorriti MA, Fernández-Ruiz JJ, Palomo T and Ramos JA (1994a) Down regulation of rat brain cannabinoid binding sites after chronic δ^9-tetrahydrocannabinol treatment. *Pharmacol. Biochem. Behav.*, **47**, 33–40.

Rodríguez de Fonseca, F.A., Martín-Calderón, J.L., Del Arco, I., Gorriti, M.A., Navarro, M. (1998) Role of the endogenous cannabinoid system in the regulation of motor activity. *Neurobiology of Disease* **5**, 483–501.

Rodríguez de Fonseca F, Martin-Calderón JL, Mechoulam R and Navarro M (1994b) Repeated stimulation of D-1 dopamine receptors enhances (-)-11-hydroxy-Δ^8-tetrahydrocannabinol-dimethylheptyl-induced catalepsy in male rats. *NeuroReport*, **5**, 761–765.

Rodríguez de Fonseca, F.; Wenger, T., Navarro, M.; Murphy, L.L. (1999) Effects of THC on VIP-induced prolactin secretion in pituitary cultures: evidence for the presence of functional cannabinoid receptors in pituitary cells. *Brain Res.* **841**, 114–122.

Romero J, García-Palomero E, Fernández-Ruiz JJ, and Ramos JA (1995) Involvement of GABA-B receptors in the motor inhibition produced by agonists of brain cannabinoid receptors. *Behav. Pharmacol.*, **7**, 299–302.

Sañudo-Peña MC, Patrick SL, Patrick RL and Walker JM (1996) Effects of intranigral cannabinoids on rotational behavior in rats: interactions with the dopaminergic system. *Neurosci. Lett.*, **206**, 21–24.

Sañudo-Peña MC, and Walker JM (1997) Role of the subthalamic nucleus in cannabinoid actions in the substantia nigra of the rat. *J. Neurophysiol.*, **77**, 1635–1638.

Sañudo-Peña, M.C. and Walker, J.M. (1998). Effects of intrapallidal cannabinoids on rotational behavior in rats. Interaction with the dopaminergic system. Synapse 28, 2–32.

Sim, L.J., Selley, D.E., Dworkin, S.I. and Childers, S.R. (1996) Effects of chronic morphine administration on mu opioid receptor-stimulated [35S]GTPgS autoradiography in rat brain. J. Neurosci., 16, 2684–2692.

Steiner, H., Bonner, T.I., Zimmer, A.M., Kitai, S.T. and Zimmer, A. (1999) Altered gene expression in striatal projection neurons in CB1 cannabinoid receptor knockout mice. *Proc. Natl. Acad. Sci.* **96**, 5786–5790.

Stella N, Schweitzer P and Pomelli D (1997) A second endogenous cannabinoid that modulates long-term potentiation. *Nature*, **388**, 773–777.

Surmeier DJ, Song WJ and Yan Z (1996) Coordinated expression of dopamine receptors in neostriatal medium spiny neurons. *J. Neurosci.*, **16**, 6579–6591.

Tanda, G, Pontieri, FE, Di Chiara, G (1997) Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common mul opioid receptor mechanism. *Science* **76**, 2048–2050.

Tersigni T and Rosemberg HC (1996) Local pressure application of cannabinoid agonists increases spontaneous activity of rat substantia nigra pars reticulata neurons without affecting response to iontophoretically-applied GABA. *Brain Res.*, **733**, 184–192.

Tsou, K., Sañudo-Peña, M.C., Mackie, K., Walker, J.M. (1998) Immunohystochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. *Neuroscience* **83**, 393–411.