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European Journal of Pharmacology 559 (2007) 180–183
www.elsevier.com/locate/ejphar

Short communication

Cannabinoid CB_1 receptor antagonism markedly increases dopamine receptor-mediated stereotypies

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Received 31 August 2006; received in revised form 18 December 2006; accepted 8 January 2007
Available online 19 January 2007

Abstract

The contribution of the endocannabinoid system to dopamine-mediated disorganized behavior in schizophrenia is discussed. We used a model of concurrent stimulation of dopamine D_1 and D_2 receptors to evaluate the role of this system in dopamine-mediated stereotypies measured in a hole-board test. Pretreatment with the cannabinoid CB_1 receptor antagonist *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichloro-phenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (SR141716A; 1 mg/kg) potentiated stereotyped behavior induced by coadministration of the dopamine D_1 receptor agonist SKF 38393 (0.05, 0.1 and 1 mg/kg) and the dopamine D_2 receptor agonist quinpirole (0.25 mg/kg). Thus, the endocannabinoid system acts as a brake for abnormal behavior associated with dopaminergic overactivation.

Keywords: Endocannabinoids; Cannabinoid receptors; Dopamine receptors; Schizophrenia; Stereotypies

1. Introduction

Cannabis preparations act in the brain through the activation of cannabinoid CB_1 receptors, disrupting physiological endogenous cannabinoid signaling (Rodriguez de Fonseca et al., 1998). Debate exists concerning the potential psychiatric consequences of the acute and chronic use of cannabis. Several studies have reported the benefits on negative symptoms of cannabis use by schizophrenic patients (Peralta and Cuesta, 1992), whereas longitudinal studies suggested that the acute and chronic consumption of this drug is associated with an increased risk for the onset of psychosis (Andreasson et al., 1987; Rodriguez De Fonseca et al., 2001). A potential site for cannabinoid action is the mesotelencephalic dopaminergic circuits where cannabinoid CB_1 receptors are heavily expressed. The endogenous cannabinoid system has been found to modulate dopamine signaling in these mesotelencephalic networks involved in motor control, emotional responses or cognitive processes (Giuffrida et al., 1999; Rodriguez de Fonseca et al., 1998). As an example, in the dorsal striatum anandamide release stimulated by the activation of dopamine D_2 receptors acts as a negative feedback signal that limits behavioral activation elicited by dopamine (Giuffrida et al., 1999). The dopamine D_1 receptor also participates since administration of a CB_1 receptor agonist is able to attenuate the dyskinesias induced by dopaminergic D_1 agonists in a Parkinson's disease model (Ferrer et al., 2003).

However, many gaps remain in our knowledge of the pathophysiological role of dopamine–cannabinoid interactions (Rodriguez De Fonseca et al., 2001). The analysis of these

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doi:10.1016/j.ejphar.2007.01.009

relationships may help to understand the contribution of the endogenous cannabinoid system to the pathogenesis of dopamine-related neuropsychiatric disorders such as schizophrenia, where a clinical correlation exists between cerebrospinal fluid level of endocannabinoids, positive symptoms and responsiveness to dopamine D_2 receptor preferring neuroleptics (Giuffrida et al., 2004). In this regard, previous reports from our laboratory suggest that the cannabinoid CB_1 receptor acts as a brake for dopamine-mediated behavioral activation, as suggested by the enhanced motor responses to both amphetamine and to the dopamine D_2 receptor agonist quinpirole, found after desensitization of the cannabinoid CB_1 receptor (Gorriti et al., 1999, 2005). Since dopamine-mediated behavioral disorganization, including stereotypies, are considered positive symptoms in schizophrenia, we studied whether cannabinoid CB_1 receptors modulate dopamine-mediated stereotypies. We tested this hypothesis in rats by using a model of combined dopamine D_1 and D_2 receptor stimulation in animals pretreated with a cannabinoid CB_1 receptor blocker, and studied stereotyped behavior in a hole-board test.

2. Materials and methods

2.1. Animals

Male Wistar rats (Panlab, Barcelona, Spain) weighing 350± 35 g at the start of the experiment were housed individually and maintained in a temperature- and light-controlled environment on a 12-h light/dark cycle (lights on: 08:00–20:00) with free access to food and water. Animals were allowed at least a 2-week period for acclimatization to the animal room. They were subsequently handled daily for a week before the beginning of the experimental sessions. All the procedures were carried out according to the European Communities Directive of 24 November 1986 (86/609/EEC) regulating animal research. All the experiments took place between 10:00 and 13:00.

2.2. Drugs

N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichloro-phenyl)-4-methyl-1*H*-pyrazole-3-carboxamide, SR141716A (cannabinoid CB_1 receptor antagonist) was generously donated by Sanofi-Aventis (France). It was suspended in saline/propylenglycol/Tween 80 [90:5:5 v/v] as vehicle and made up to the appropriate concentrations (0.1 and 1 mg/kg) to be administered i.p. in a volume of 1 ml/kg. The dopamine D_2 receptor agonist quinpirole hydrochloride (0.25 mg/kg) and the dopamine D_1 receptor agonist SKF 38393 (0.05, 0.1 and 1 mg/kg) were provided by Research Biochemicals International as part of the Chemical Synthesis Program of the US National Institute of Mental Health, contract N01MH30003. They were dissolved in saline and injected s.c. in a final volume of 0.5 ml/kg.

2.3. Behavioral testing

We developed a model of dopamine receptor-mediated behavioral overactivation by concurrent stimulation of dopa-

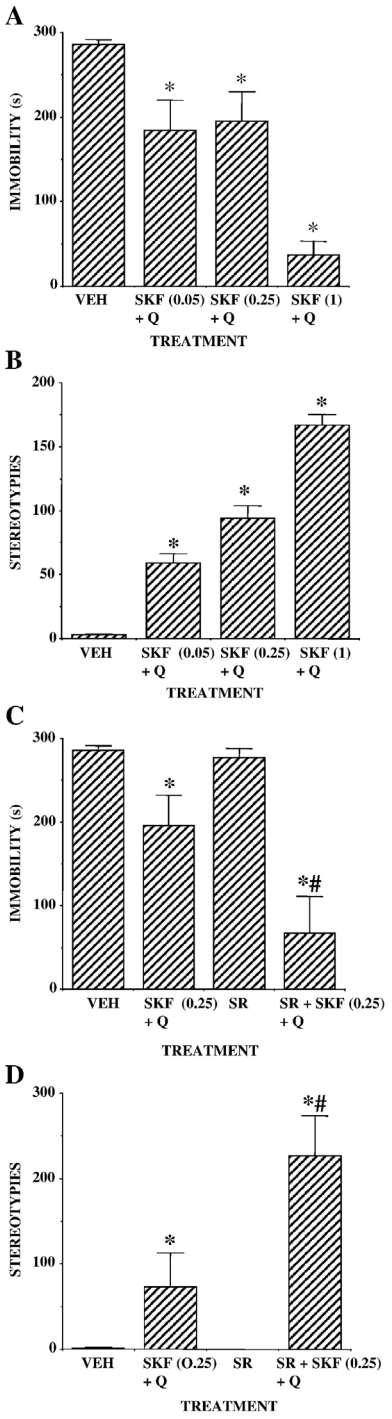

Fig. 1. Simultaneous administration of a fixed dose of the dopamine D_2 receptor agonist quinpirole (Q, 0.25 mg/kg) with increasing doses of the dopamine D_1 agonist SKF 38393 (SKF, 0.05, 0.25 and 1 mg/kg) produced both dose-dependent hyperactivity, as reflected in the decreasing time spent in the absolute immobility (A), and disorganized behavior, as reflected in the increasing stereotyped activity (B). Blockade of cannabinoid CB1 receptors with SR141716A (SR, 1 mg/kg) markedly enhanced the response to quinpirole (0.25 mg/kg) plus SKF (0.25 mg/kg), increasing the general activity (C) and the stereotyped behavior (D). Data are means±S.E.M. of 8–10 determinations per group. (*) $P<0.01$ different doses versus vehicle-treated animals and (#) $P<0.01$, SR+SKF+Q versus SKF+Q, Newman Keul's test.

mine D_1 and D_2 receptors, in accordance with previous reports (Dall'Olio et al., 1988; Gorriti et al., 1999; Longoni et al., 1987). The study was performed with a hole-board apparatus, similar to that described previously (Gorriti et al., 1999). It consisted of a 0.5×0.5 m square open field. Evenly spaced on the floor were 25 holes arranged in five parallel rows of five holes each. Four black lines painted on the floor divided the apparatus into 25 equal square sectors. The field was surrounded by vertical opaque walls of 40 cm height. The field was illuminated with a halogen ceiling light, adjusted to yield 350 lx at the center of the field. The test was initiated by placing the rat in the center of the field. Animals were placed in the apparatus 5 min after the injection of the dopaminergic agonists and observed during four consecutive intervals of 5 min each, every 30 min. The following behavioral acts were scored by trained observers blind to experimental conditions: *immobility* was defined as the time spent by the animals without moving — this is a marker of behavioral activation (Gorriti et al., 1999) and *stereotypies* were repetitive behavioral acts, counted without considering the initial act — this is a marker of dopamine-mediated behavioral disorganization. According to this model, a theoretical sequence of 5 dippings in hole 1, followed by a dip in hole 2, a rearing, and two dips in hole 6 will be scored as three dips plus a rearing and 5 units of stereotyped behavior (5 repetitive dippings).

2.4. Drug treatment and experimental design

Following previous reports, we performed a full dose–response study of the interactions between dopaminergic agonists on behavioral disorganization. Fixed doses of quinpirole (0.025, 0.25 or 1 mg/kg) were combined with increasing doses of SKF 38393 (0.05, 0.25 and 1 mg/kg). The administration of a low dose of quinpirole (0.025 mg/kg) barely affected SKF 38393-induced behavioral activation, whereas the administration of quinpirole at 1 mg/kg in combination with SKF 38393 produced aberrant behavior, with compulsive biting and self-mutilation. This schedule was therefore discarded for future experiments (data not shown). However the combination of quinpirole at 0.25 mg/kg with increasing doses of SKF 38393 produced a dose-dependent stereotyped behavior and was selected as the schedule for the experiments with the cannabinoid receptor blocker. In order to test the influence of cannabinoid CB_1 receptor blockade we selected the doses of 0.1 and 1 mg/kg, injected 30 min prior to the administration of the combination of quinpirole plus SKF 38393.

3. Results

Combined treatment with the D_2 dopaminergic agonist quinpirole (0.25 mg/kg) and the growing doses of the dopamine D_1 receptor agonist SKF 38393 (0.05, 0.25 and 1 mg/kg) resulted in dose-dependent behavioral activation (locomotion, sniffing, head movements and rearing) reflected in the decrease in the total time spent in absolute immobility (Fig. 1A). This behavioral activation was clearly associated with the appearance of stereotypies, which were dose-dependently induced by increasing doses of the dopaminergic D_1 receptor agonist when injected simultaneously with quinpirole (0.25 mg/kg). The dopamine D_1 receptor agonist alone did not induce stereotyped behavior (Fig. 1B and data not shown). To analyze the effects of the blockade of cannabinoid CB_1 receptors on behavioral activation induced by this combination of dopaminergic agonists, we selected the intermediate combination of quinpirole (0.25 mg/kg) and SKF 38393 (0.25 mg/kg) to avoid ceiling effects. While pretreatment with a low dose of SR141716A (0.1 mg/kg) did not affect the response to this combination of dopaminergic agonists (Table 1), pretreatment with a dose of SR141716A that blocks central cannabinoid CB_1 receptors (1 mg/kg) resulted in a robust behavioral activation (Fig. 1C) and produced a marked increase in stereotypies (over 2 fold, Fig. 1D), which were even more frequent than those observed after maximal stimulation with dopamine D_1 and D_2 receptor agonists (Fig. 1B).

Table 1
Administration of a low dose of the cannabinoid CB_1 receptor antagonist SR141716A (SR, 0.1 mg/kg) did not enhance the response to the dopamine D_1 receptor agonist SKF 38393 (SKF, 0.25 mg/kg) coadministered with the dopamine D_2 receptor agonist quinpirole (Q, 0.25 mg/kg) on the time spent on immobility(s) and the number of stereotypies

	Vehicle	SR	SKF+Q	SR+SKF+Q
Immobility(s)	235±12	255±13	154±36[a]	124±28[a]
Stereotypies	2.9±0.7	1.1±0.4	93.7±1[a]	125±10[a]

Data are means±S.E.M. of 8–10 determinations per group.
[a] $P<0.01$ treatment versus vehicle-treated animals, Newman Keul's test.

4. Discussion

As previously described (Dall'Olio et al., 1988; Longoni et al., 1987; White et al., 1988), combined stimulation of dopamine D_1 and D_2 receptors resulted in dose-dependent behavioral activation associated with stereotypies. This pharmacological response is elicited in the dorsal and ventrolateral striatum, as demonstrated by local injections of both types of dopaminergic agonists (Bordi and Meller, 1989; Delfs and Kelley, 1990). This basal ganglia area contains a dense presence of cannabinoid CB_1 receptors that regulate synaptic input and output from the medium spiny GABAergic neurons, the projecting neurons of the striatum (Mato et al., 2004; Rodriguez de Fonseca et al., 1998, 2001). Dopamine input from the substantia nigra is a relevant modulatory neurotransmitter that has a permissive role in movement initiation, sequencing and ending. Overactivation of dopamine transmission is associated with aberrant behaviors and stereotypies, a marker of psychostimulant-induced disorganized behavior (Gorriti et al., 1999). This abnormal motor behavior is also observed in acute schizophrenia, and it is considered a positive symptom of the disease. Dopamine-mediated disorganized behavior is limited by local regulatory signals, among which the locally released endocannabinoids seem to play a key role. Dopamine can stimulate anandamide release through the activation of both dopamine D_1 and D_2 receptors (Ferrer et al., 2003; Giuffrida et al., 1999).

Anandamide, by engaging the cannabinoid CB_1 receptor, reduces dopamine-mediated behavioral activation. The present study extends this notion to disorganized-stereotyped behavior and reveals that cannabinoid receptor blockade boosts stereotyped behavior stimulated by combined administration of dopaminergic agonists. A potential explanation for this phenomenon is the removal of the endocannabinoid brake to dopaminergic overactivation. A similar finding has been described in cannabinoid CB_1 receptor knockout mice, which displayed more stereotypies after receiving an acute dose of the NMDA antagonist phencyclidine (a pharmacological model of schizophrenia; Haller et al., 2005). A similar explanation to that seen in dopaminergic circuits may explain these results, because the endocannabinoids act as retrograde messengers limiting synaptic output at glutamatergic synapses. These findings support the hypothesis of a potential role for the endocannabinoid system as a relevant neurotransmitter in the pathogenesis of behavioral disorganization typical of psychosis. Although studies in humans are contradictory concerning a direct role for cannabis consumption in schizophrenia (Giuffrida et al., 2004; Peralta and Cuesta, 1992), the present study gives further support to the contribution of local regulatory mechanisms controlled by cannabinoid CB_1 receptors to dopamine-mediated disorganized behavior. Further research in humans is needed to understand to what extent this neurobiological mechanism may be useful for the control or prevention of psychotic episodes.

Acknowledgements

This study was supported by Plan Nacional Sobre Drogas, Instituto de Salud Carlos III, grants Redes Temáticas FIS Red CIEN (C03/06) and Red de Trastornos Adictivos (05/2540), and MEC grants SAF 2003-4864, Grant FIS and GEN 2003-20651.

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