Cannabidiol is a partial agonist at dopamine D2High receptors, predicting its antipsychotic clinical dose

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Although all current antipsychotics act by interfering with the action of dopamine at dopamine D2 receptors, two recent reports showed that 800 to 1000 mg of cannabidiol per day alleviated the signs and symptoms of schizophrenia, although cannabidiol is not known to act on dopamine receptors. Because these recent clinical findings may indicate an important exception to the general rule that all antipsychotics interfere with dopamine at dopamine D2 receptors, the present study examined whether cannabidiol acted directly on D2 receptors, using tritiated domperidone to label rat brain striatal D2 receptors. It was found that cannabidiol inhibited the binding of radio-domperidone with dissociation constants of 11 nM at dopamine D2High receptors and 2800 nM at dopamine D2Low receptors, in the same biphasic manner as a dopamine partial agonist antipsychotic drug such as aripiprazole. The clinical doses of cannabidiol are sufficient to occupy the functional D2High sites. It is concluded that the dopamine partial agonist action of cannabidiol may account for its clinical antipsychotic effects.

Original Article

INTRODUCTION

Although currently used antipsychotics for schizophrenia all interfere with the neurotransmission of dopamine,1 a report by Leweke et al.2 showed that cannabidiol at 800 mg/day was as clinically effective as amisulpride in alleviating the signs and symptoms of schizophrenia. In addition, a recent report by P. McGuire et al.3 found that 1000 mg per day cannabidiol added to the ongoing antipsychotic treatment significantly improved schizophrenia in a study on 88 patients. Because there are no known reports of cannabidiol acting directly on dopamine D2 receptors, the important findings by Leweke et al.2 and McGuire et al.3 may indicate that cannabidiol is the first apparent exception to the general rule that all antipsychotics either block or interfere with dopamine at brain dopamine D2 receptors.4,5

In fact, McGuire et al.3 went so far as to state that the antipsychotic target for cannabidiol was not dopaminergic. Furthermore, the report by Leweke et al.2 did not attribute the antipsychotic action of cannabidiol to any particular set of receptors in the brain. For example, it is possible that cannabidiol may act on one or more types of receptors to exert its clinical action. Such possible targets include fatty acid amide hydrolase, serotonin-1 receptors, GPR55 receptors and transient potential vanilloid-1 receptors.6–8

Cannabidiol is an active cannabinoid found in high concentrations in the cannabis plant (up to 40% in the extract). Cannabidiol has many possible uses in a variety of medical illnesses, especially in certain types of epilepsy and possibly in treating psychosis.9

Although dopamine D2 receptors are a main common target for antipsychotic drugs, it was essential, therefore, for this present study to examine whether cannabidiol had any direct action on dopamine D2 receptors that might account for the clinical antipsychotic effects observed by McGuire et al.3 and Leweke et al.2 Such an investigation was considered essential in order to test the basis for the commonly known dopamine hypothesis of psychosis,5 especially as it is known that the potent cannabinoïds HU210 and Win 55,212-2 cause behavioral dopamine supersensitivity and elevated D2High receptors.5 That is, should there be no effect of cannabidiol on dopamine D2 receptors, despite having a clinical antipsychotic action, the dopamine hypothesis underlying psychosis would need to be modified.

MATERIALS AND METHODS

Tissue preparation

Rat striatal tissues were used as a source of dopamine D2 receptors. The rat striata were from carbon-dioxide-killed Sprague-Dawley rats or from frozen rat brains (Pel-Freez Biologicals, Rogers, AR, USA). The brain (stored at –70°C) was partly thawed and the striata removed. The striata were homogenized in buffer (4 mg frozen tissue per ml buffer), using a teflon-glass homogenizer (with the piston rotating at 500 r.p.m.) and 10 up-and-down strokes of the glass container. The buffer contained 50 mM Tris-HCl (pH 7.4 at 20°C), 1 mM EDTA, 5 mM KCl, 1.5 mM CaCl2, 4 mM MgCl2 and 120 mM NaCl. The homogenate was not washed, centrifuged or preincubated because our previous work found that 30–50% of the D2 receptors were lost by these procedures.10

Cannabidiol/[3H]domperidone competition

The dopamine D2 receptors in the rat striatal tissue were measured with [3H]domperidone (2 to 3 nM final concentration in the incubation tube; custom-synthesized as [phenyl-3H(N)]domperidone; 41.4 Ci mmol−1; made by Moravek Biochemicals and Radiochemicals, Brea, CA, USA). Each incubation tube (12 x 75 mm, glass) received, in the following order, 0.5 ml buffer (with or without a final concentration of 10 μM S-sulpiride to define nonspecific binding to the dopamine D2 receptors; and with or without 10 μM guanilylimidodiphosphate), 0.25 ml [3H]domperidone, and 0.25 ml of tissue homogenate. The tubes (total volume of 1 ml contents) were incubated for 2 h at room temperature (20°C), after which the incubates were filtered using a 12-well cell harvester (Titertek, Skatron, Lier, Norway) and buffer-presoaked glass fiber filters (Whatman GF/C). After filtering the radioactive filters, the samples were shaken for 1 h and counted in a scintillation counter.
St. Louis, MO, USA). and compounds were obtained from commercial sources (Sigma-Aldrich, domperidone (that is, Scatchard plot) to the striatal homogenate. Reagents 

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concentrations (usually 2.3 nM) that occupied about cannabidiol concentrations from 0.1 to 10,000 nM, using [3H]fraction of D2 receptors occupied was also used to derive the dissociation constants (K_i values) were 11 nM at D2High and 2800 nM at D2Low.

Furthermore, in the presence of 200 μM guanilylimidodiphosphate, the binding of [3H]domperidone to the high-affinity site (at D2High receptors) was completely abolished. All the dopamine D2High receptors had converted to their D2Low state in the presence of the guanine nucleotide.

In contrast to the reproducible biphasic pattern of cannabidiol competition versus the binding of [3H]domperidone, there was no such biphasic competition when cannabidiol competed against the binding of [3H]raclopride (data not shown). In fact, cannabidiol inhibited the binding of [3H]raclopride with only a single dissociation constant of 4900 nM at the dopamine D2Low receptors (with an IC_{50}% concentration of 9000 nM). Such a phenomenon was also previously seen with dopamine, which competed in a biphasic manner versus [3H]domperidone but in a monophasic manner at D2Low receptors when using [3H] raclopride. 

**DISCUSSION**

The data in Figure 1 clearly show that cannabidiol inhibited the binding of [3H]domperidone in two phases, corresponding to dopamine D2High and D2Low dopamine receptors. This biphasic pattern only occurs with dopamine agonists, as consistently found with hallucinogens and the commonly used anti-Parkinson drugs. Such a biphasic pattern does not occur with any of the antipsychotics, except for aripiprazole, which is a partial agonist at the dopamine D2 receptors, similar to the clinical antipsychotic action of aripiprazole.

Brexpiprazole and cariprazine are relatively new compounds and have not yet been examined in this assay.

Therefore, the biphasic pattern of cannabidiol in inhibiting the binding of [3H]domperidone, in exactly the same way as aripiprazole, indicates that cannabidiol may act clinically as a partial agonist at the dopamine D2 receptors, similar to the clinical antipsychotic action of aripiprazole.

The present data for cannabidiol may help explain some effects of cannabidiol that has actions similar to atypical antipsychotic drugs. Such a partial agonist-type action at a G-protein-linked receptor has been reported for delta9-tetrahydrocannabinol at the rat cerebellar cannabinoid receptor but not for cannabidiol. In fact, it was reported that cannabidiol behaved as an antagonist in the micromolar concentration range.

The present data for cannabidiol acting at the dopamine D2High sites with a dissociation constant of 11 nM may well account for the antipsychotic effects reported by McGuire et al. and Leweke et al., because the dopamine D2High receptors are considered to be the functional dopamine D2 receptor sites. More specifically, the clinical daily doses of 800 mg and 1000 mg cannabidiol would adequately occupy the D2High receptors in vivo. For example, a daily dose of 800 mg cannabidiol would result in an extracellular free concentration level in humans of the order of 600 nM, after allowing for at least 99% of the cannabidiol to be bound to plasma proteins.

In addition, considering that cannabidiol has a K_i value of 11 nM at D2High, as compared with 0.2 nM for aripiprazole, a possible clinical antipsychotic dose for cannabidiol would be of the order of 55-fold higher than aripiprazole. Because the antipsychotic dose of aripiprazole is between 10 and 20 mg per day, the
antipsychotic dose for cannabidiol would be 550 to 1100 mg per day, which is the dose range that Leweke et al.2 and that McGuire3 used for patients with schizophrenia.

It may be argued that the present in vitro effect of cannabidiol may occur by the action of cannabidiol on the striatal cannabinoid CB1 receptors that are colocalized as heteromers with dopamine D2 receptors,24,25 thereby indirectly influencing the binding of [3H]dopaminedione at the D2 receptors. However, the following important points indicate that such an indirect action through CB1/D2 heteromers is unlikely.

Cannabidiol concentrations between 35 and 350 nM effectively inhibited the binding of [3H]dopaminedione to the D2High site (Figure 1). However, the dissociation constant of cannabidiol at the brain cannabinoid CB1 receptors is between 4350 and 350 nM,9 indicating that the CB1 receptors would not be significantly affected by cannabidiol concentrations between 35 and 350 nM.

Moreover, although the expression of CB1/D2 heteromers is found in 30 to 60% of monkey striatal neurons,26 only a very small non-significant influence was found for the alteration of dopamine competition of the D2 ligand [3H]YM-09151-02 on caudate nucleus membranes by the CB1 agonist CP55940, thereby indicating a very weak interaction between CB1 and D2 receptors.26

It is not known why cannabidiol inhibited the binding of [3H]dopaminedione in a biphasic manner at D2High and D2Low receptor sites (Figure 1), yet inhibited the binding in a monophasic manner at only D2Low when using [3H]raclopride (data not shown). A similar situation occurred with dopamine,14 as mentioned above. It is possible that the two different [3H]ligands have a different mode of attachment to the dopamine D2 receptor, based on the fact that dopaminedione has a pKa of 7.9, whereas raclopride has a pKa of 8.97, resulting in a different proportion of charged and uncharged species of the two ligands, where the charged nitrogen atom of dopaminedione and raclopride is expected to be the main point of attachment. The fact that [3H]raclopride was not competed by cannabidiol at D2High precludes the ready measurement of cannabidiol versus the binding of [11C] raclopride in possible studies in humans.

The apparent partial agonist action of cannabidiol in vitro in the present work may possibly account for some of the clinical side-effects of cannabis such as somnolence, diarrhea, decreased appetite and fatigue.27 Moreover, because it is well known that the partial dopamine agonist aripiprazole can sometimes elicit psychotic signs and symptoms in patients,28 it is entirely possible that long-term use of marijuana can lead to an accumulation of the cannabidiol partial agonist in some individuals and cause a psychotic episode. This is unlikely, however, because cannabidiol has been reported to protect against the effects of tetrahydrocannabinol.29,30

CONFLICT OF INTEREST
The author declare no conflict of interest.

ACKNOWLEDGMENTS
I thank Dr H.-C. Guan for excellent technical assistance and Dr Bertha K. Madras and Dr Harold Kalant for helpful science advice. This work was supported by Janet Marsh Frosst, David Medland, Pamela and Desmond O’Rorke, in memory of John William Medland, and by the estate of the late Dr Karolina Jus.

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