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Agonistic Properties of Cannabidiol at 5-HT1a Receptors

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Cannabidiol (CBD) is a major, biologically active, but psycho-inactive component of cannabis. In this cell culture-based report, CBD is shown to displace the agonist, [3H]8-OH-DPAT from the cloned human 5-HT1a receptor in a concentration-dependent manner. In contrast, the major psychoactive component of cannabis, tetrahydrocannabinol (THC) does not displace agonist from the receptor in the same micromolar concentration range. In signal transduction studies, CBD acts as an agonist at the human 5-HT1a receptor as demonstrated in two related approaches. First, CBD increases [35S]GTPγS binding in this G protein coupled receptor system, as does the known agonist serotonin. Second, in this GPCR system, that is negatively coupled to cAMP production, both CBD and 5-HT decrease cAMP concentration at similar apparent levels of receptor occupancy, based upon displacement data. Preliminary comparative data is also presented from the cloned rat 5-HT2a receptor suggesting that CBD is active, but less so, relative to the human 5-HT1a receptor, in binding analyses. Overall, these studies demonstrate that CBD is a modest affinity agonist at the human 5-HT1a receptor. Additional work is required to compare CBD's potential at other serotonin receptors and in other species. Finally, the results indicate that cannabidiol may have interesting and useful potential beyond the realm of cannabinoid receptors.

KEY WORDS: Cannabis; cannabidiol; cAMP; G Proteins; marijuana; serotonin; THC.

INTRODUCTION

Although cannabis and its extracts have been extensively studied, knowledge of the biochemical mechanisms of one of its major components, cannabidiol (CBD), has not been thoroughly explored (1,2). This lack of knowledge of CBD's biochemical pharmacology is noteworthy in the context of its known potential in human therapy: for example, it has been demonstrated to have anxiolytic (3), anti-seizure (4), anti-psychotic (3), and neuroprotective properties (5,6). While previously thought to be sedating, recent clinical research has confirmed that CBD is activating, and that it counters sedative effects of THC (7).

The major psychoactive component of cannabis, tetrahydrocannabinol (THC), has received extensive research attention into its biochemical pharmacology. Both THC and CBD have been pharmacologically investigated at cannabinoid receptors (CBR), which are highly conserved across animal taxa, with the major exception of insects (8–10). THC is at least 10 times more potent in binding to CB1 receptors than CB2 receptors. At CB1R, there is evidence to suggest that CBD is an antagonist or inverse agonist, although substantial debate still exits about its intrinsic activity (10,11). CBD has received little attention in other neurotransmitter systems. Noteworthy in this regard is serotonin (5-hydroxytryptamine; 5-HT), which is known to be involved in many of the same processes important to cannabis's actions (12,13) such as relief of anxiety, pain, the complex processes of

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headache (14,15), and thermoregulation. The few studies done with CBD in serotonergic systems suggest that it inhibits 5-HT re-uptake, and overall reduces 5-HT neurotransmission (2,16). There is also some experimental evidence to support CBD's activity in other neurotransmitter systems such as dopamine, GABA, and the endogenous opioid system (2).

Most of 5-HT's broad actions are thought to be regulated at a series of 5-HT receptors (5-HTR), the majority of which (17) are members of the diverse super family of G-protein coupled (GPC), seven-transmembrane receptors (7TMR). The 5-HT1aR (17) has been cloned and studied in numerous *in vivo* and cell culture systems and in various species. It has been cloned in both human (H) and rat (18–20), amongst other organisms, and has been further analyzed in other species, including rabbit (21), where it has not been cloned. In this literature, extending over two decades, 5-HT1aR has been ever more implicated in a variety of physiological and pathological processes including anxiety, mood, depression, panic, obsessive-compulsive disorders, headache, immune regulation, and cardiovascular regulation to name a few (2,6,17,18). Additionally, the 5-HT2aR could have relevance to the pharmacology of cannabis as it has been associated with phenomena like mood, headache, and hallucination (22). There is precedence for the action of cannabinoids such as oleamide at serotonin receptors (23–26).

Over the last decade our laboratory has conducted a series of studies with 5-HT1aR (27), and to a lesser extent with 5-HT2aR (21). Because of these interests and our hypothesis that CBD may have important actions relevant to the pharmacology of cannabis but outside the realm of CBR, we report here studies with H5-HT1aR and a limited comparison to the rat 5-HT2aR (28). For both H5-HT1aR and rat 5-HT2aR we also report comparisons between CBD and THC. In cell culture experiments with cloned human 5-HT1aR and rat 5-HT2aR, CBD has a greater affinity than THC for both receptors. CBD binds with higher affinity at 5-HT1aR than at 5-HT2aR. In the case of H5-HT1aR, CBD appears to act as an agonist. A preliminary report of these investigations has appeared (29).

EXPERIMENTAL PROCEDURE

Cell Culture. Chinese Hamster Ovary (CHO) cells expressing the H5-HT1aR (19) were cultured in Ham's F-12 medium fortified with 10% fetal calf serum and 200 ug/ml geneticin. Cultures were maintained at 37°C in a humidified atmosphere of 5% CO2. Cells were sub-cultured or assayed upon confluency (5–8 days). Cloned H5-HT1aR was kindly provided by Dr. John Raymond (Medical U. of South Carolina). NIH 3T3 cells expressing the rat 5-HT2aR (28) were cultured under similar conditions in DMEM fortified with 10% calf serum and 200 μg/ml geneticin. These transfected cells were generously provided by Dr. David Julius (UCSF). Both cell lines have been tested for mycoplasma with a PCR kit (ATCC), and are free of contamination.

Receptor Preparation. Cells were harvested by trypsinization and centrifuged at low speed in ice-cold medium. The pellet was re-suspended in ice-cold Earle's Balanced Salt Solution followed by centrifugation. Cells were re-suspended in 10 ml of ice-cold binding buffer (50 mM Tris, 4 mM CaCl2, 10 μM pargyline, pH 7.4), homogenized with Teflon-glass, and centrifuged for 450,000 g-min. at 4°C. To produce a crude membrane preparation, the pellet was re-suspended in 30 ml of ice-cold binding buffer, and homogenized, first with Teflon-glass and then with a Polytron (setting 4) for 5 s. The receptor preparation was stored on ice and assayed within the next 1.5 h.

Assay of Receptor Activity. Binding of the agonist [3H]8-OH-DPAT ([3H]8-hydroxy-2-(di-n-propylamino)tetralin) to H5-HT1aR followed well-characterized *in vitro* protocols (20,27,30). Radioligands were purchased from New England Nuclear (NEN), Boston, MA. 1 ml reaction mixtures, in triplicate, were incubated for 30 min. in a 30°C shaker bath. Composition of the 1 ml reaction mixture was: 700 μl of receptor preparation; 100 μl of either binding buffer (for total binding) or 10 μM 5-HT (final concentration for non-specific binding), 100 μl of the tritiated agent (final concentration of 0.5 nM [3H] 8-OH-DPAT), and 100 μl of diluted CBD or binding buffer in the case of controls.

Reactions were stopped by addition of 4 ml of ice-cold 50 mM Tris buffer, pH 7.4, and subsequent vacuum filtration on glass fiber filters (Whatman GF/B). Filters were rinsed twice in 5 ml of ice-cold Tris buffer, dried, and counted in 5 ml of Ecoscint (National Diagnostics) liquid scintillation fluid in a Beckman LS 6500 instrument. Homogenates were assayed for protein to maintain a nominal value of 50 μg protein per filter over weekly assays (31). Total and non-specific binding tubes were run in triplicate. Assays of the rat 5-HT2aR (28) were conducted under similar conditions with the 1 ml reaction mixture containing: 700 μl of receptor preparation; 100 ul of either binding buffer (for total binding) or 10 μM mianserin (final concentration for non-specific binding); 100 μl of the tritiated agent (final concentration of 0.2 nM [3H] ketanserin); and 100 μl of diluted CBD or binding buffer in case of controls.

cAMP Assay. CHO cells were cultured to confluency in 12- or 24-well plates (27). Medium was aspirated and the cells were rinsed twice in warm, serum-free F-12 medium. Cells were then incubated for 20 min. at 37°C in 0.5 mls of serum-free F-12 medium containing 100 μM isobutylmethylxanthine (IBMX) and the following substances (final concentrations) alone or in combination (see Fig. 3): 30 μM forskolin (FSK; for all treatments); 1 μM 5-HT; 16 μM CBD; and 0.05 μM NAN-190 (NAN). Reactions were stopped by aspiration of medium and addition of 0.5 ml of 100 mM HCl. After 10 min., well contents were removed and centrifuged at 4000 rpm. Supernatants were diluted in 100 mM HCl, and cAMP was quantified (27) directly in a microplate format by colorimetric enzyme immunoassay (EIA) with a kit from Assay Designs (Ann Arbor). Triplicate independent samples were assayed in quadruplicate to increase precision.

[35S]GTPγS Assay. H5-HT1aR membranes from transfected CHO cells were incubated with 5-HT (0.1 μM) and/or CBD