Neurochemical Research, Vol. 30, No. 8, August 2005 (© 2005), pp. 1037–1043
DOI: 10.1007/s11064-005-6978-1

Agonistic Properties of Cannabidiol at 5-HT1a Receptors

Ethan B. Russo,[1] Andrea Burnett,[1] Brian Hall,[1] and Keith K. Parker[1,2]

(Accepted June 27, 2005)

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

[1] Skaggs School of Pharmacy, The University of Montana, Missoula, MT 59812-1552, USA.
[2] Address reprint requests to: Keith K. Parker, Skaggs School of Pharmacy, The University of Montana, Missoula, MT 59812-1552, USA. Tel.: +406-243-4235; Fax: +406-243-5228; E-mail: keith.parker@umontana.edu

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

(16 μM); see Fig.2), and the following incubation mixture: 20 mM HEPES buffer, pH 7.4, 5 mM MgCl2, 1 mM EDTA, 1 mM DTT, 100 mM NaCl, 100 uM GDP, 10 μM pargyline, 0.2 mM ascorbate, and 0.1 nM [35S]GTPγS (32). Mixtures were incubated for 30 min. at 30°C, and were terminated by dilution in cold buffer. The mixture was filtered on GF/C filters, rinsed twice in buffer, followed by drying and liquid scintillation counting. Negative control (basal incorporation) was the above mixture minus CBD or 5-HT. Non-specific binding was determined in the presence of cold GTPγS-(10 μM). Positive control was H5-HT1aR membranes in the same incubation mixture plus 5-HT. All values reported in Fig. 2 are for specific binding (total – non-specific) of triplicates.

Dilution of Cannabinoids. CBD and THC were obtained in dilute (1 mg/ml) solution from Sigma Chemical Co. (St. Louis, MO). These solutions were stored at 4°C until use and then diluted in distilled water and finally in the buffer appropriate to the particular assay. Fresh dilutions of cannabinoids were made daily. Each final concentration of cannabinoid thus contained some of the vehicle (methanol). The highest concentration of methanol encountered in any assay (1%) was then tested in that assay system for activity. In pair-wise comparative *t* testing, none of the methanol controls were found to be distinguishable from negative control (buffer).

Statistical Analysis. All statistics (means, standard deviations, standard errors of the mean (SEM), and *t* tests) were performed with software provided by Poly Software International; in some cases, statistics were corroborated by hand using a Hewlett-Packard Graphing Calculator, HP48. Graphs were constructed with Excel software provided by Microsoft.

RESULTS

Cannabidiol produces concentration-dependent displacement of the agonist [3H[8-OH-DPAT from the H5-HT1aR (Fig. 1). Using crude membrane preparations from cultured CHO cells transfected with H5-HT1aR (See methods), CBD diluted in methanolic buffer displaced agonist by 73 ± 8 % (S.E.M.) at 16 μM. The highest concentration of methanol (1%) present at 32 μM CBD produced only 3 ± 0.5% displacement of agonist, a level indistinguishable from control when the methanol and control means are compared statistically. While CBD was active in the micromolar range, tetrahydrocannabinol was unable (108 ± 6% of control) to produce agonist displacement at a concentration of 32 μM.

The ability of CBD to produce concentration-dependent displacement of highly potent and specific agonist from the H5-HT1aR ligand-binding site raised the question of the intrinsic activity of CBD. Experiments were designed to test the agonistic potential of cannabidiol. Since H5-HT1aR is G protein-coupled, agonist binding would be expected to increase GTP binding, measurable when the stable analog of GTP, GTP γS is present in a radiolabeled form. 0.1 μM 5HT increased [35S]GTPγS incorporation by 57 ± 7% (Fig. 2) above the basal level (buffer) in membranes of CHO transfected with the receptor. Similarly, 16 μM CBD increased [35S]GTPγS incorporation by 67 ± 6% above the basal level. Together, 5-HT and CBD increased [35S]GTPγS incorporation to 123 ± 10% above the basal level, suggesting that CBD had not reached its maximum possible stimu-

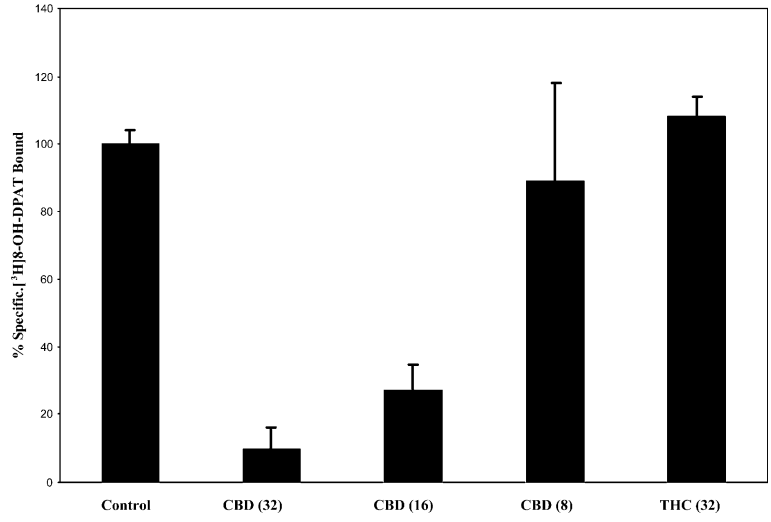

Fig. 1. Displacement of Specifically-Bound [3H]8-OH-DPAT By Cannabidiol (CBD) and Tetrahydrocannabinol (THC) In Membranes Containing the Human 5-HT1a Receptor. Concentrations are micromolar. Values are the mean ± SEM with n's = 3–6. More detailed experimental conditions of cell culture, membrane preparation, and drug-receptor binding are outlined in Experimental Procedure.

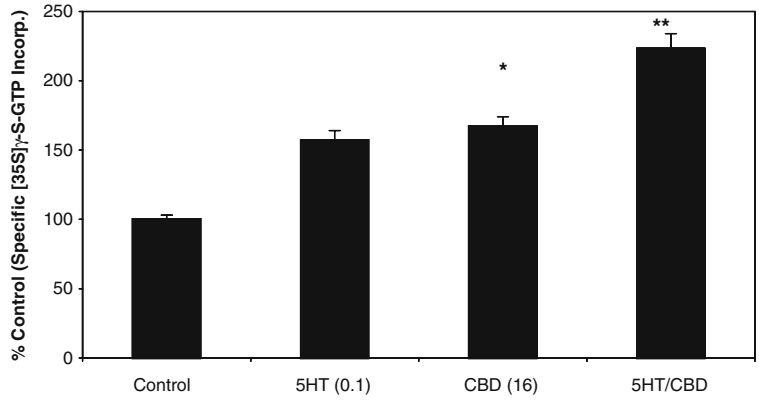

Fig. 2. Incorporation of [35S]GTPγS by Cannabidiol (CBD) In Membranes Containing the Human 5-HT1a Receptor. Control represents incorporation in the basal setting (buffer). Concentrations in micromolar are: 5-HT (0.1); CBD (16). Results are expressed relative to basal incorporation as mean ± SEM with n's = 9–18. *$P < 0.01$, relative to Control;**$P < 0.01$, relative to 5HT. Further experimental details are found in Experimental Procedure.

lation. By reference to CBD's displacement capacity at the receptor's ligand binding site (Fig. 1), 16 μM CBD occupies about 73% of the available binding sites.

To further test the hypothesis that CBD is an agonist at H5-HT1aR, experiments were designed to measure activity in the adenylyl cyclase (AC) system negatively coupled to the receptor. In this format, AC is first stimulated by the natural product forskolin (FSK) at a concentration of 30 μM (control = 100 ± 5%). 1 μM of the agonist 5-HT reduced FSK-stimulated cAMP to 29 ± 8% of control (Fig. 3). 16 μM CBD reduced FSK-stimulated cAMP to 38 ± 3% of control. At a concentration of 0.05 μM, the highly specific 5-HT1aR antagonist NAN-190 reduced the 5-HT effect to 60 ± 7% of control and the CBD effect to 76 ± 5% of control, providing further evidence that CBD is acting at the ligand- binding site of H5-

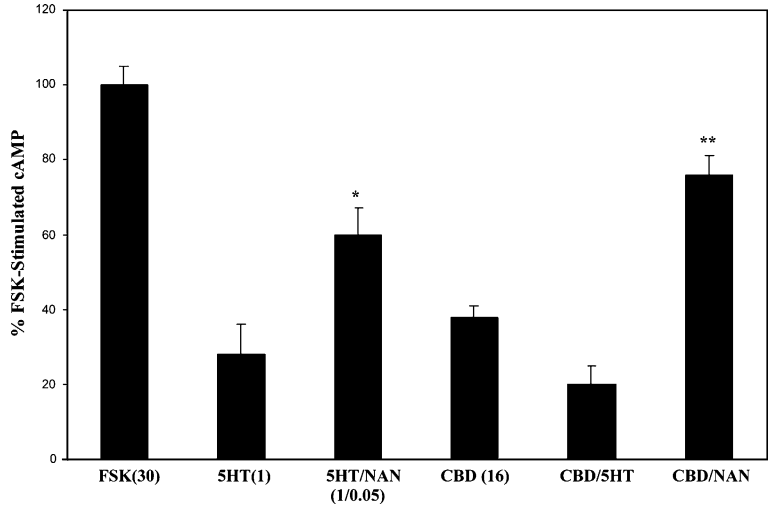

Fig. 3. Inhibition of Forskolin (FSK)-Stimulated cAMP by Cannabidiol (CBD), Serotonin (5-HT), and the inhibitor NAN-190 (NAN) in Whole Cells Transfected With the Human 5-HT1a Receptor. All conditions contain FSK at 30 μM and the phosphodiesterse inhibitor isobutylmethylxanthine (IBMX) at 100 μM. Other concentrations in micromolar are: 5-HT (1); CBD (16); and NAN (0.05). Results are expressed as percentage of FSK control as mean ± SEM with n's = 3–6. *$P < 0.05$, relative to 5-HT; **$P < 0.01$, relative to CBD. Further experimental details are found in Experimental Procedure.

HT1aR. At the concentration used here (0.05 μM), NAN-190 does not reduce FSK-stimulated cAMP levels on its own (data not shown).

Since the 5-HT2aR is another receptor putatively involved in the pathogenesis of migraine headache, we conducted a limited comparison at cloned rat 5-HT2aR. At the highest concentration of CBD tested (32 μM), 50 ± 5% of [3H]Ketanserin is displaced from membrane preparations of the cloned rat 5-HT2aR. The displacement is concentration-dependent as lower concentrations of CBD progressively displace less ketanserin, until at 8 μM CBD, the effect is barely above control level. Comparatively, then, CBD is less potent in displacement from the rat 5-HT2aR relative to H5-HT1aR. As with H5-HT1aR, THC (32 μM) is inactive in displacement from rat 5-HT2aR. Signal transduction properties of CBD at rat 5-HT2aR have not been explored yet.

DISCUSSION

There is substantial literature to support the idea that tetrahydrocannabinol (THC) is responsible for many of the meaningful and diverse components of cannabis' pharmacological activity (33), but other available evidence supports important contributions of CBD and other phytocannabinoids and terpenoids to its pharmacological activity (34,35). It is well established that the pharmacology of cannabis combines therapeutic properties (e.g., benefits on neuropathic pain and spasticity) (36–39), and lower urinary tract symptoms (40) that must be weighed against adverse effects such as intoxication that may be counter-productive in a therapeutic sense. A prominent example of the latter is the hallucinogenic potential of cannabis demonstrated at higher doses, especially in certain cultural settings. There is also an outstanding body of experimental evidence to suggest that THC is hallucinogenic while the closely related cannabinoid, cannabidiol (CBD) opposes such activity (3,41).

In pursuit of those pharmacological actions of cannabis that may underlie some of its medicinally important possibilities, differentiation between THC and CBD at the receptor level may be of significance. This could be especially so at non-cannabinoid receptors such as 5-HT receptors. The results shown in Fig. 1 establish such a contrast in that CBD shows micromolar affinity in displacing a known agonist, [3H]8-OH-DPAT, from the 5-HT1aR ligand-binding site, THC is inactive in the same concentration range.

CBD's 5-HT1aR potency could underlie activity anywhere along the intrinsic activity continuum from full agonist to silent antagonist. Experiments summarized in Figs. 2 and 3 provide evidence that CBD is likely to behave as an agonist in this receptor system. Thus, CBD demonstrated the ability to increase GTP binding to the receptor coupled G protein, Gi, which is characteristic behaviour of a receptor agonist. These GPCR are further linked to effector signal transduction sub-systems such as, in the case of a Gi GPCR, the AC step in cAMP regulation. In Fig. 3, when AC is stimulated by forskolin (FSK), the agonist 5-HT markedly reduces cAMP production in this negatively coupled complex. Likewise, CBD acts as an agonist in these experiments by reducing cAMP concentration. The results in Figs. 2 and 3 together support the hypothesis that CBD is an agonist. Although not completely conclusive in demonstrating whether CBD is a full or partial agonist, the comparable power of CBD and 5-HT at concentrations that represent less than full receptor occupancy (Fig. 1) lend support to the full agonist concept.

The contrast between CBD and THC regarding their interactions at 5-HT1aR relative to CB1R is striking. THC is at least 10 times more potent in binding to CB1R; at 5-HT1aR the relationship is just the opposite, where CBD has micromolar affinity, and THC shows no binding in the micromolar range. At CB1R, THC has sub-micromolar affinity, yet CBD has micromolar affinity. The comparison continues into the realm of signal transduction, where at CB1R, CBD is putatively an antagonist or inverse agonist (2); at 5-HT1aR, we have concluded that CBD is an agonist.

What implications do these results at 5-HT1aR have for CBD and cannabis? Cannabis is a very complex mixture of chemical compounds (42), as is true of most crude natural product drug mixtures. The dearth of biochemical investigations with non-psychoactive cannabis components, such as CBD, create a void of understanding regarding the use of one or more of these pharmacologically active components as therapeutic agents. It has recently been demonstrated that CBD stimulates TRPV1 (one of the vanilloid receptors), inhibits the reuptake of anandamide, and weakly inhibits its hydrolysis (42), thus making it possibly the first pharmacotherapeutic agent to modulate endocannabinoid function (1). As anandamide has already shown activity at 5-HT1aR, and 36% inhibition of function at 5-HT2aR (14), the

psychopharmacological importance of such relationships is underscored.

The results reported here argue that CBD is active as an agonist *in vitro* at H5-HT1a R and that CBD may also have *in vitro* actions at the rat 5-HT2aR. Should CBD prove to have antagonistic activity at 5-HT2A, it would support its role as a migraine prophylactic agent (19). Together, these results lend credence to the idea that CBD and related compounds merit study at a variety of receptor systems, in a number of species, and at various levels from the molecular to whole animal. If, for example, CBD demonstrates clinical activity at 5-HT1aR *in vivo*, therapeutic possibilities could arise in a variety of neurological and other physiologically relevant settings.

ACKNOWLEDGMENTS

The authors would like to thank the following individuals for their assistance and ideas that contributed to this project: Rustem Medora, Alicia Christians, Cortney Halley, Sonja Sakaske, Ben Seaver, and Lynn Parker . The following agencies are gratefully acknowledged for their financial support of the work: NIH NIGMS grants #: GM/OD 54302–01 and 02 and NIH NCRR grant #: P20 RR 15583 to the NIH COBRE Center for Structural and Functional Neuroscience.

REFERENCES

1. Russo, E. B. 2003. Introduction: Cannabis: from pariah to prescription. J. Cann. Therap. 3(3–4):1–29.
2. Pertwee, R. G. 2004. The pharmacology and therapeutic potential of Cannibidiol. Pages 1–52, in DiMarzo, V. (ed.), Cannabinoids. Kluwer Academic/Plenum Publishers.
3. Zuardi, A. W. and Guimaraes, F. S. 1997. Cannabidiol as an anxiolytic and antipsychotic. Pages 133–141, in Mathre, M. L. (ed.), Cannabis in medical practice: a legal, historical and pharmacological overview of the therapeutic use of marijuana. NC: McFarland: Jefferson.
4. Carlini, E. A. and Cunha, J. M. 1981. Hypnotic and antiepileptic effects of cannabidiol. J. Clin. Pharmacol. 21(8–9 Suppl):417S–427S.
5. Hampson, A. J., Grimaldi, M., Axelrod, J., and Wink, D. 1998. Cannabidiol, (-)Delta9-tetrahydrocannabinol are neuroprotective antioxidants. Proc. Natl. Acad. Sci. USA. 95(14):8268–73.
6. Iuvone, T., Esposito, G., Esposito, R., Santamaria, R., Di Rosa, M., and Izzo, A. A. 2004. Neuroprotective effect of cannabidiol, a non-psychoactive component from cannabis sativa, on beta-amyloid-induced toxicity in PC 12 cells. J. Neurochem. 89:134–141.
7. Nicholson, A. N., Turner, C., Stone, B. M., and Robson, P. J. 2004. Effect of delta-9-tetrahydrocannabinol and cannabidiol on nocturnal sleep and early-morning behavior in young adults. J. Clin. Psychopharmacol. 24(3):305–313.
8. McPartland, J., Di Marzo, V., De Petrocellis, L., Mercer, A., and Glass, M. 2001. Cannabinoid receptors are absent in insects. J. Comp. Neurol. 436(4):423–9.
9. Pertwee, R.G. 1997. ''Pharmacology of cannabinoid CB1 and CB2 Receptors''. Pharmacol. Therap. 74(2):129–180.
10. Thomas, B. F., Gilliam, A. F., Burch, D. F., Roche, M. J., and Seltzman, H. H. 1998. ''Comparative receptor binding analysis of cannabinoid agonists and antagonists''. J. Pharm. Exp. Therap 285(1):285–292.
11. Petitet, F., Jeantaud, B., Reibaud, M., Imperato, A., and Dubroeueq, M. C. 1998. ''Complex pharmacology of natural cannabinoids: Evidence for partial agonist activity at delta 9-tetrahydrocannabinol and antagonist activity of cannabidiol on rat brain cannabinoid receptors''. Life Sci. 63 (1):PL1–PL6.
12. McPartland, J. M. and Russo, E. B. 2001. Cannabis and Cannabis Extracts: Greater Than the Sum of Their Parts? J. Cannabis Therap. 1(3–4):103–132.
13. Russo, E. B. 2001. Hemp for headaches: An in-depth historical and scientific review of cannabis in migraine treatment. J. Cannabis Therap. 1(2):21–92.
14. Russo, E. B. 2004. Clinical endocannabinoid deficiency (CECD): Can this concept explain therapeutic benefits of cannabis in migraine, fibromyalgia, irritable bowel syndrome and other treatment-resistant conditions? Neuroendocrinol. Lett. 25(1–2):31–39.
15. Ferrari, M. D. and Saxena, P. R. 1995. 5-HT1 receptors in migraine pathophysiology and treatment. Eur. J. Neurol. 2:5–21.
16. Hershkowitz, M. 1978. ''The effect of *in vivo* treatment with (-)/delta 1-tetrahydrocannabinol, and other psychoactive drugs on the *in vitro* uptake of biogenic amines''. Adv. Biosci. 22–23:351–358.
17. Cowen, P. J. 2000. Psychopharmacology of 5-HT1a receptors. Nuc. Med. Biol. 27:437–439.
18. Barnes, N. M. and Sharp, T. 1999. A review of central 5-HT receptors and their function. Neuropharmacol. 38(8):1083–1152.
19. Fargin, A., Raymond, J. R., Lohse, M. J., Kobilka, B. K., Caron, M. G., and Lefkowitz, R. J. 1988. The genomic clone G-21 which resembles a beta-adrenergic receptor sequence encodes the 5-HT1a receptor. Nature 335:358–360.
20. Nelson, D. L., Monroe, P. J., Lambert, G., and Yamamura, H. I. 1987. [3H]Spiroxatrine labels a serotonin 1a-like site in rat hippocampus''. Life Sci. 41:1567–1576.
21. Weber, J. T., O'Connor, M.-F., Hayataka, K., Colson, N., Medora, R., Russo, E. B., and Parker, K. K. 1997. Activity of parthenolide at 5HT2a receptors. J. Nat. Prods. 60(6):651–653.
22. Glennon, R. A., Teitler, M., and Sanders-Bush, E. 1992. Hallucinogens and serotonergic mechanisms. NIDA Res. Monographs 119:131–135.
23. Hedlund, P. B., Carson, M. J., Sutcliffe, J. G., and Thomas, E. A. 1999. Allosteric regulation by oleamide of the binding properties of 5-hydroxytryptamine 7 receptors. Biochem. Pharmacol. 58:1807–1813.
24. Boger, D. L., Patterson, J. E., and Jin, Q. 1998. Structural requirements for 5-HT2A and 5-HT1A serotonin receptor potentiation by the biologically active lipid oleamide. Proc. Natl. Acad, Sci, USA 95(8):4102–4107.
25. Cheer, J. F., Cadogan, A. K., Marsden, C. A., Fone, K. C., and Kendall, D. A. 1999. ''Modification of 5HT2a receptor

mediated behaviour in the rat by oleamide and the role of cannabinoid receptors''. Neuropharmacol. 38(4):533–545.
26. Devlin, M. G. and Christopoulos, A. 2002. ''Modulation of cannabinoid agonist binding by 5-HT in the rat cerebellum''. J. Neurochem. 80(6):1095–1102.
27. Ortiz, T. C., Devereaux, M. C., and Parker, K. K. 2000. Structural variants of a human 5-HT1a receptor intracellular loop 3 peptide. Pharmacol. 60:195–202.
28. Julius, D., Huang, K. N., Livelli, T. J., Axel, R., and Jessel, T. M. 1990. The 5HT2 receptor defines a family of structurally distinct but functionally conserved serotonin receptors''. Proc. Natl. Acad. Sci.USA 87:928–932.
29. Hall, B., Burnett, A., Halley, C., Parker, L., Russo, E., and Parker, K. K. 2004. Pharmacology of cannabidiol at serotonin receptors. Proc. West. Pharmacol. Soc. 47:43–(M24).
30. Pierce, P. A. and Peroutka, S. J. 1989. Hallucinogenic drug interactions with neurotransmitter receptor binding sites in human cortex''. Psychopharmacol. 97:118–122.
31. Bradford, M. M. 1976. A rapid sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248–254.
32. Wieland, T. and Jacobs, K. H. 1994. Measurement of receptor-stimulated guanosine 5'-O-(gamma-thio)triphosphate)binding by G proteins. Meth. Enzymol. 237:3–27.
33. Wachtel, S. R., ElSohly, M. A., Ross, R. A., Ambre, J., and de Wit, H. 2002. Comparison of the subjective effects of delta9-tetrahydrocannabinol and marijuana in humans. Psychopharmacology 161:331–339.
34. McPartland, J. M. and Russo, E. B. 2001. Cannabis and cannabis extracts: Greater than the sum of their parts? J. Cann. Therap. 1(3–4):103–132.
35. Russo, E. B. and McPartland, J. M. 2003. Cannabis is more than simply Delta(9)-tetrahydrocannabinol. Psychopharmacology (Berl.) 165(4):431–2.
36. Berman, J. S., Symonds, C., and Birch, R. 2004. Efficacy of two cannabis based medicinal extracts for relief of central neuropathic pain from brachial plexus avulsion: results of a randomized controlled trial. Pain 112(3):299–306.
37. Notcutt, W., Price, M., Miller, R., Newport, S., Phillips, C., Simmonds, S., and Sansome, C. 2004. Initial experiences with medicinal extracts of cannabis for chronic pain: results from 34 ''N of 1'' studies. Anaesthesia 59:440–452.
38. Wade, D. T., Makela, P., Robson, P., House, H., and Bateman, C. 2004. Do cannabis-based medicinal extracts have general or specific effects on symptoms in multiple sclerosis? A double-blind, randomized, placebo-controlled study on 160 patients. Mult. Scler. 10(4):434–41.
39. Wade, D. T., Robson, P., House, H., Makela, P., and Aram, J. 2003. A preliminary controlled study to determine whether whole-plant cannabis extracts can improve intractable neurogenic symptoms. Clin. Rehabil. 17:18–26.
40. Brady, C. M., DasGupta, R., Dalton, C., Wiseman, O. J., Berkley, K. J., and Fowler, C. J. 2004. An open-label pilot study of cannabis based extracts for bladder dysfunction in advanced multiple sclerosis. Mult. Scler. 10:425–433.
41. Karniol, I. G., Shirakana, I., Kasinski, N., Pfeferman, A., and Carlini, E. A. 1974. ''Cannabidiol Interferes With the Effects of Delta 9-Tetrahydrocannabinol in Man''. Eur. J. Pharmacol. 28(1):172–177.
42. Bisogno, T., Hanus, L., De Petrocellis, L., Tchilibon, S., Ponde, D. E., Brandi, I., Moriello, A. S., Davis, J. B., Mechloulam, R., and DiMarzo, V. 2001. Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. Br. J. Pharmacol. 134(4):845–852.
43. Howlett, A.C. 2002. ''International union of pharmacology XXVII classification of cannabinoid receptors''. Pharmacol. Rev. 54(2):161–202.