THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 271, No. 17, Issue of April 26, pp. 9902–9905, 1996
© 1996 by The American Society for Biochemistry and Molecular Biology, Inc.
Printed in U.S.A.

Communication

(−)-Δ^9-Tetrahydrocannabinol Antagonizes the Peripheral Cannabinoid Receptor-mediated Inhibition of Adenylyl Cyclase*

(Received for publication, March 1, 1996)

Michael Bayewitch, Man-Hee Rhee, Tomer Avidor-Reiss, Aviva Breuer, Raphael Mechoulam, and Zvi Vogel‡

From the Department of Neurobiology, The Weizmann Institute of Science, Rehovot 76100, Israel and the Department of Natural Products, Faculty of Medicine, Hebrew University of Jerusalem, Jerusalem 91120, Israel

(−)-Δ^9-Tetrahydrocannabinol ((−)-Δ^9-THC) is the major active psychotropic component of the marijuana plant, *Cannabis sativa*. The membrane proteins that have been found to bind this material or its derivatives have been called the cannabinoid receptors. Two GTP-binding protein-coupled cannabinoid receptors have been cloned. CB1 or the neuronal cannabinoid receptor is found mostly in neuronal cells and tissues while CB2 or the peripheral cannabinoid receptor has been detected in spleen and in several cells of the immune system. It has previously been shown that activation of CB1 or CB2 receptors by cannabinoid agonists inhibits adenylyl cyclase activity. Utilizing Chinese hamster ovary cells and COS cells transfected with the cannabinoid receptors we report that (−)-Δ^9-THC binds to both receptors with similar affinity. However, in contrast to its capacity to serve as an agonist for the CB1 receptor, (−)-Δ^9-THC was only able to induce a very slight inhibition of adenylyl cyclase at the CB2 receptor. Morever, (−)-Δ^9-THC antagonizes the agonist-induced inhibition of adenylyl cyclase mediated by CB2. Therefore, we conclude that (−)-Δ^9-THC constitutes a weak antagonist for the CB2 receptor.

Most of the original cannabinoid receptor research was devoted to the analysis of the function of the cannabinoid receptor designated CB1 due to its earlier cloning and the large amount of work performed on neuronal cells and tissues, which contain this receptor (1–6). Activation of CB1 leads to inhibition of adenylyl cyclase (AC)[1] (1) in various brain tissues and neuronal cells as well as to the inhibition of N-type voltage-dependent calcium channels in a number of *in vitro* systems (3–6). (−)-Δ^9-THC, the active cannabinoid compound from *Cannabis sativa*, has been shown to be a potent agonist for this receptor and to initiate various receptor-mediated biochemical and behavioral responses (5–9).

Although the original focus of cannabinoid function was on the nervous system, it has been noted that there are specific binding sites for cannabinoid ligands in non-neuronal tissues and cells (1, 10). Cannabinoid binding sites have been localized to regions of the mouse and rat spleen, and it has been proposed that these sites are located on myeloid cells, B-cells, or mast cells (10–12). Indeed, a second cannabinoid receptor, designated CB2, has recently been cloned from the HL-60 promyelocytic leukemia cell line (13). Like CB1, it belongs to the seven-transmembrane GTP-binding protein (G protein)-coupled receptor family and was shown to be able to bind (−)-Δ^9-THC as well as various (−)-Δ^9-THC derivatives (13–15).

Since (−)-Δ^9-THC is the active ingredient of marijuana, which is popularly used as a mood-altering drug by many human subjects, we and others have investigated the effect of (−)-Δ^9-THC on the signal transduction of the CB1 receptor (2, 3, 5, 7, 8). It was shown that (−)-Δ^9-THC inhibits AC in neuronal cells (*e.g.* NG108–15 neuroblastoma × glioma and $N_{18}TG_2$ neuroblastoma cells) as well as in CB1-transfected cell lines (5, 7, 8, 16, 17). Since cannabinoids also have effects on immunological functions (18–21), which may be mediated by the CB2 receptor, it was of interest to define the activity of (−)-Δ^9-THC on this receptor. Here we show that although many cannabinoid agonists inhibit AC activity through the activation of CB2 (14, 15, 22), (−)-Δ^9-THC showed a very weak agonistic activity. Moreover, (−)-Δ^9-THC reversed the effects obtained with other cannabinoid agonists of the CB2 receptor.

EXPERIMENTAL PROCEDURES

Materials—HU-210, (−)-Δ^9-THC, HU293a, and [^{3}H]HU-243 have been described previously (15, 23–25). Cannabinoids were kept in ethanol and diluted before use in 50 mg/ml fatty acid-free bovine serum albumin as described (23). The phosphodiesterase inhibitors, 1-methyl-3-isobutylxanthine and RO-20–1724, were from Calbiochem. Forskolin (FSK) and cAMP were from Sigma. The plasmids, pCD containing rat CB1 (2), pCDM8 containing human CB2 (13), and pXMD1 containing the cDNA for AC type V (26), were kindly provided by Drs. T. Bonner (NIH, Bethesda, MD), S. Munro (Cambridge, UK), and T. Pfeuffer (Düsseldorf, Germany), respectively.

Cell Cultures—Chinese hamster ovary (CHO) cells stably transfected with CB2 receptor were described earlier (15). COS-7 cells were obtained from ATCC (Bethesda, MD) and $N_{18}TG_2$ from Dr. Nirenberg (NIH, Bethesda, MD). Cell lines were cultured in Dulbecco's modified Eagle's medium supplemented with 8% fetal calf serum, 2 mM glutamine, nonessential amino acids, 100 units/ml penicillin, and 100 μg/ml streptomycin in a humidified atmosphere consisting of 5% CO_2 and 95% air at 37 °C.

Transfection of COS Cells—COS-7 cells in 10-cm dishes were transfected by the DEAE-dextran chloroquine method (27) with CB1 or CB2 plasmids (5 μg each) and, when indicated, with 2 μg of the plasmid containing the cDNA of AC type V. Twenty-four hours later, the cells were trypsinized and cultured in 24-well plates. After an additional 24 h, the cells were assayed for AC activity. Transfection efficiency, determined by transfection with the cDNA for β-galactosidase, was 40–80%.

Receptor Binding Assay—COS-7 cells were transfected with 5 μg/dish CB1 or CB2 cDNA. Two days after transfection, the cells from each dish were washed 2 times with phosphate-buffered saline, scraped, and stored at −80 °C. Cell pellets were homogenized in 2 ml of binding buffer (50 mM Tris-HCl, 5 mM $MgCl_2$, and 2.5 mM EDTA, pH 7.4). Aliquots of 50 μg of protein were placed into siliconized Eppendorf tubes. The labeled cannabinoid ligand [^{3}H]HU-243 (54 Ci/mmol) was added (300 pM) together with unlabeled cannabinoid competitors, and binding was determined (8, 23). The K_i values for (−)-Δ^9-THC were calculated from the competition data according to the formula K_i =

* This work was supported by grants from the National Institute on Drug Abuse (DA6481 and DA6265), the Israeli Academy of Sciences and Humanities, and the Forschheimer Center for Molecular Genetics. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed. Tel.: 972-8-934-2402; Fax: 972-8-934-4131.

[1] The abbreviations used are: AC, adenylyl cyclase; CHO, Chinese hamster ovary; (−)-Δ^9-THC, (−)-Δ^9-tetrahydrocannabinol; G proteins, GTP-binding proteins; FSK, forskolin.

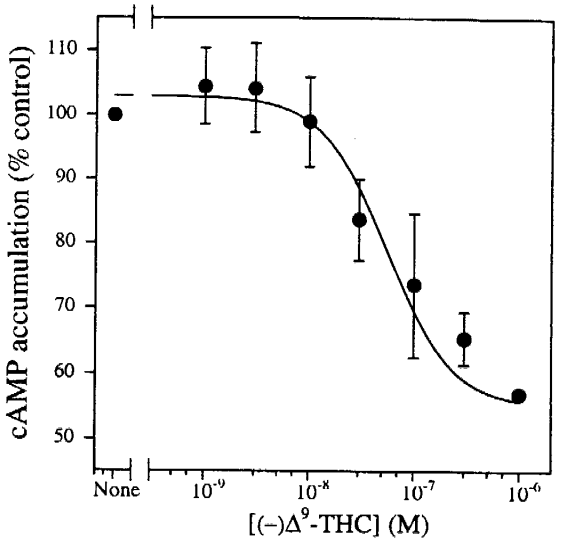

FIG. 1. **(−)-Δ⁹-THC inhibits the FSK-stimulated AC activity in $N_{18}TG_2$ neuroblastoma cells.** 100% cAMP accumulation represents AC activity in the absence of (−)-Δ⁹-THC and is equivalent to 4540 ± 315 cpm. Data are means ± S.E. of two independent experiments performed in duplicate.

$IC_{50}/1 + ([^3H]HU\text{-}243/K_d)$ (28). The K_d values for HU-243 binding were 45 (23) and 61 pM (15) for CB1 and CB2, respectively.

Adenylyl Cyclase Assay—The assay was performed as described (8, 15). Cells cultured in 24-well plates were incubated for 3 h with 0.25 ml/well fresh growth medium containing 5 μCi/ml [³H]adenine. This medium was replaced with Dulbecco's modified Eagle's medium containing 20 mM Hepes (pH 7.4), 1 mg/ml fatty acid-free bovine serum albumin, 0.1 mM 1-methyl-3-isobutylxanthine, and 0.5 mM RO-20-1724. Cannabinoids and FSK (1 μM) were added and the cells incubated at 37 °C for 10 min. The reaction was terminated with 1 ml of 2.5% perchloric acid containing 0.1 mM unlabeled cAMP. Aliquots of 0.9 ml were neutralized with 100 μl of 3.8 M KOH and 0.16 M K_2CO_3 and applied to a two-step column separation procedure (29). The [³H]cAMP was eluted into scintillation vials and counted. Background levels (cAMP accumulation in the absence of FSK) were subtracted from all values and represented less than 10% of FSK-stimulated cAMP accumulation.

Statistical Analysis—Data were analyzed using the Student's *t* test. Inhibition curves were generated with the Sigma Plot 4.11 program, and the EC_{50} values were determined using an equation from the ALLFIT program (30).

RESULTS

(−)-Δ⁹-THC Inhibits AC in Cells That Express CB1 but Not in Cells That Express CB2—It has been reported that (−)-Δ⁹-THC is a fairly active agonist of the CB1 receptor (2, 5, 7, 8). As a control experiment (see Fig. 1) we show that (−)-Δ⁹-THC inhibits FSK-stimulated AC activity in $N_{18}TG_2$ neuroblastoma cells, known to express the CB1 receptor (2, 7, 17). The EC_{50} found for (−)-Δ⁹-THC was 35 ± 7 nM, and the level of inhibition reached 45% at 1 μM (−)-Δ⁹-THC. These values are consistent with previously published results (31). Similarly, (−)-Δ⁹-THC inhibited the FSK-stimulated AC activity in COS cells transiently transfected with the cDNA of CB1 receptor (Fig. 2*A*), with an EC_{50} of 13 ± 3 nM and maximal inhibition of 62% at 1 μM (−)-Δ⁹-THC. The COS cells were cotransfected with AC type V to increase the level of AC activity and the sensitivity of the assay. Only slight inhibition of AC by (−)-Δ⁹-THC (less than 10% at 1 μM) was observed in cells that were cotransfected with the cDNAs of CB2 receptor and AC type V (see Figs. 2*A* and 3).

The difference between the results obtained with CB1- and CB2-transfected COS cells was not due to variations in the efficiency of AC or cannabinoid receptor transfection, since both groups of transfected cells were equivalently stimulated by FSK (demonstrating equivalent transfection by AC type V)

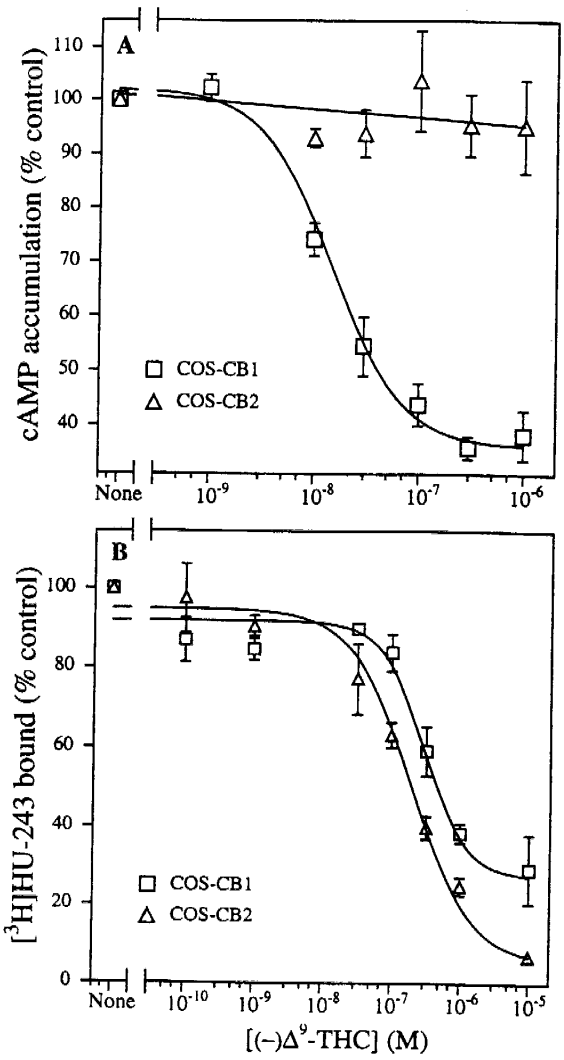

FIG. 2. **Effect of (−)-Δ⁹-THC on cAMP accumulation and [³H]HU-243 binding in COS cells transfected with CB1 or CB2 cDNA.** *A* shows the effect of increasing concentrations of (−)-Δ⁹-THC on FSK-stimulated AC activity in COS cells transfected with AC type V and CB1 or CB2 cDNAs. 100% represents cAMP accumulation in the absence of (−)-Δ⁹-THC (about 5000 cpm for both receptors). *B* shows the competition for binding of [³H]HU-243 by various concentrations of (−)-Δ⁹-THC. 100% binding represents 302 ± 8 and 305 ± 18 fmol of [³H]HU-243 bound per mg of protein to COS-CB1 and COS-CB2, respectively. Data are means ± S.E. of two independent experiments performed in duplicate.

and both showed similar levels of receptor expression, as determined by specific binding of [³H]HU-243 (see Fig. 2*B* legend). Moreover, as shown in Fig. 2*B*, (−)-Δ⁹-THC binds to both receptors on COS cells and competes with [³H]HU-243 binding with similar affinities. The K_i values of (−)-Δ⁹-THC calculated from these data are 39.5 ± 3 and 40 ± 6 nM for the CB1 and CB2 receptors, respectively. These values are comparable with those recently reported by others (14, 22). The (+) isomer of (−)-Δ⁹-THC, known to be relatively inactive on CB1 (7), showed very weak affinity for both CB1 and CB2 (data not shown) and did not inhibit AC through either of the two receptors (Fig. 3).

(−)-Δ⁹-THC Antagonism in CB2-transfected COS 7 and CHO Cells—The finding that (−)-Δ⁹-THC binds to the CB2 receptor but does not inhibit AC significantly led us to examine whether it has antagonistic properties. The cannabinoid ligand, HU-293a, inhibits AC through both CB1 and CB2 receptors (see Fig. 3 and Ref. 15). We have examined the effect of (−)-Δ⁹-THC

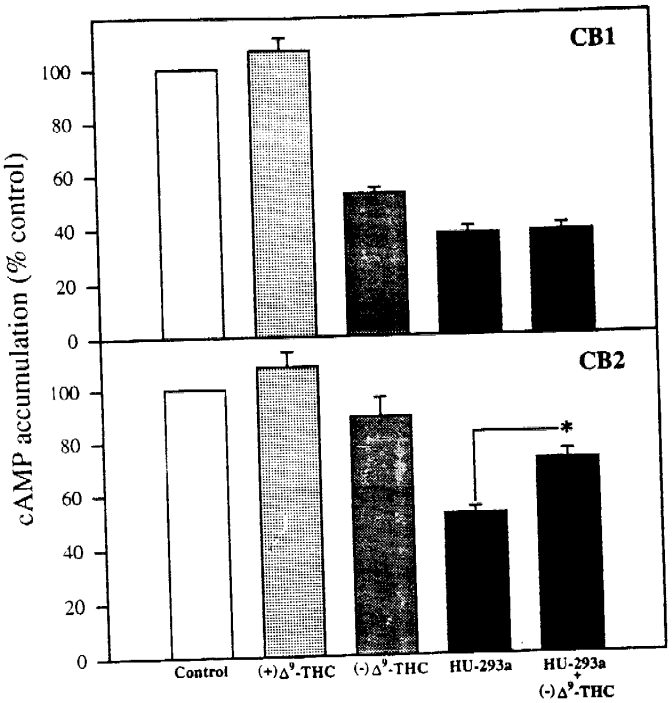

FIG. 3. **(−)-Δ⁹-THC is an efficient agonist of the CB1 but not of the CB2 receptor.** COS cells were transiently cotransfected with AC type V and CB1 or CB2 cDNAs, and the FSK-stimulated AC activity was determined in the presence of 0.1 μM of the indicated cannabinoids. The difference in AC activity observed between HU-293a and HU-293a together with (−)-Δ⁹-THC was significant according to Student's *t* test (*, $p < 0.005$). Data are means ± S.E. of four independent experiments performed in triplicate.

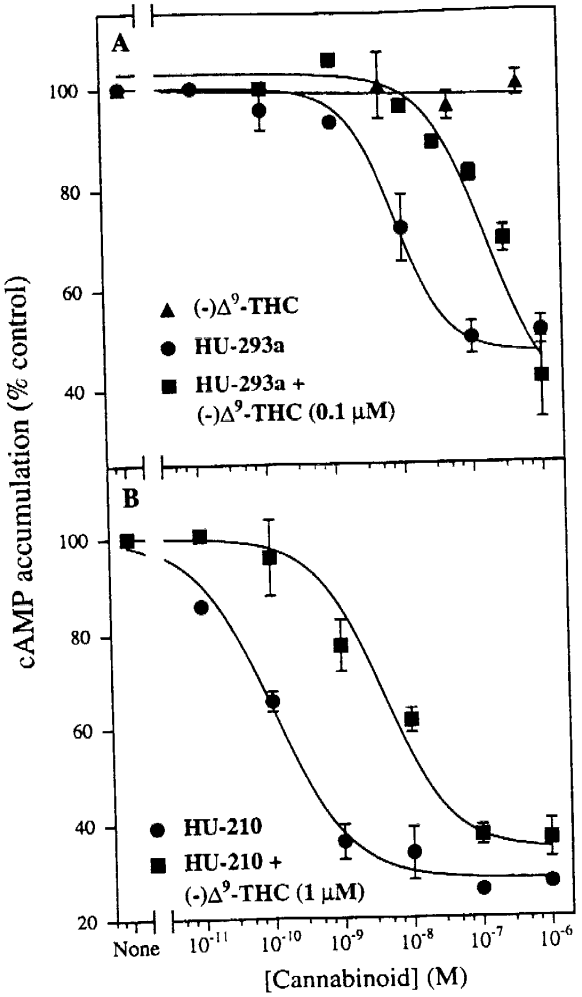

FIG. 4. **(−)-Δ⁹-THC antagonizes the capacity of the cannabinoid agonists HU-293a and HU-210 to inhibit the FSK-stimulated AC activity in CHO-CB2 cells.** The cannabinoids (−)-Δ⁹-THC and HU-293a (*A*) or HU-210 (*B*) were added at the indicated concentrations. 100% represents the amount of cAMP in the absence of cannabinoids and ranged between 1500 and 2200 cpm. Data are means ± S.E. of two to three independent experiments performed in triplicate.

on AC inhibition produced by HU-293a. As shown in Fig. 3, (−)-Δ⁹-THC partially reversed the inhibitory effect of HU-293a in COS cells expressing CB2 receptor but not in cells expressing CB1 receptor.

This phenomenon was observed not only with transiently transfected COS cells but also with CHO cells stably transfected with the CB2 receptor. Fig. 4 shows the dose-response curves for the inhibition of AC by two potent cannabinoid receptor agonists in the presence or absence of (−)-Δ⁹-THC. It shows that HU-293a and HU-210 inhibit AC with EC_{50} values of 8.2 ± 3 nM and 105 ± 1.2 pM, respectively. The EC_{50} of HU-293a in the presence of 0.1 μM (−)-Δ⁹-THC was shifted by 15-fold (to 125 ± 2 nM) and that of HU-210 in the presence of 1 μM (−)-Δ⁹-THC was shifted by 40-fold (to 4.2 ± 2 nM). This result shows that as with the COS 7-transfected cells, the activation of the CB2 receptor in CHO cells is antagonized by (−)-Δ⁹-THC.

DISCUSSION

The two members of the cannabinoid receptor family, CB1 and CB2, have been shown to share approximately 68% homology in their amino acid sequence (13). Until today, no selective agonists have been found, and no marked differences between the two receptors have been reported for agonist binding parameters (10, 13–15, 22). (−)-Δ⁹-THC is the most active psychotropic compound in *C. sativa*. Here, we demonstrate that this agonist is functionally selective in activating the CB1 but not the CB2 receptor.

(−)-Δ⁹-THC inhibits the FSK-stimulated AC activity in $N_{18}TG_2$ neuroblastoma as well as in CB1-transfected CHO or COS cells (1, 2, 7, 8, 15, 31). We and others have recently demonstrated that various cannabinoids (including HU-243, HU-210, HU-293, HU-293a, WIN 55, 212–2, and (−)CP55,940) serve as agonists of the peripheral cannabinoid receptor and inhibit AC activity (14, 15, 22). On the other hand, (−)-Δ⁹-THC did not significantly inhibit the FSK-stimulated AC activity via this receptor (15). However, (−)-Δ⁹-THC binds to the CB2 receptor with the same affinity as it binds to the CB1 receptor. Therefore, it should inhibit the action of an agonist. Here we show that (−)-Δ⁹-THC blocks the agonistic activity of other cannabinoid ligands, such as HU-293a and HU-210, on the CB2 receptor, shifting the AC inhibition curves to the right by more than 1 order of magnitude, thus serving as a partial agonist/antagonist of the CB2 receptor. It has been shown that (−)-Δ⁹-THC is chemically stable when applied to cells in culture (5). It thus seems that the antagonistic effect is due to (−)-Δ⁹-THC itself and does not result from the formation of a possible degradation product. The effect of (−)-Δ⁹-THC antagonism was dependent on the affinity of the agonist, and as agonist affinity for the receptor increased, higher concentrations of (−)-Δ⁹-THC were required to antagonize the agonist efficiently (see Fig. 4). It is of interest to note that anandamide also did not efficiently inhibit AC activity in B6C3F1 mouse splenocytes (20) or in CHO transfected with CB2 receptor (15) and that at low concentrations, it partially blocked CB1-mediated agonistic activity of (−)-Δ⁹-THC both *in vivo* and *in vitro* (32).

The difference in the response to (−)-Δ⁹-THC leads to the conclusion that it is related to intrinsic differences between the

CB1 and CB2 receptors themselves. The two cannabinoid receptors share high homology in their transmembrane domains, whereas the intracellular loops, which are known to mediate the signaling of G protein-coupled receptors (33), are significantly different (13). Line up of the two sequences demonstrates that the CB2 receptor lacks a group of 13 amino acids in its IC_3 loop as compared with CB1 (13). The difference of 13 amino acids in the IC_3 between the CB1 and CB2 receptors may affect the efficacy of receptor-G-protein coupling. In cases when the efficacy of coupling of the G-protein to the receptor is low, a weaker agonist may not be able to activate the receptor efficiently even though the ligand binds to the receptor. We previously observed that (−)-Δ^9-THC is a weaker agonist than HU-210 or HU-293a (15). Consequently, this could serve as the basis for the functional selectivity observed for (−)-Δ^9-THC. Similar situations have recently been reported for partial agonists of the muscarinic receptor (34, 35). Additional differences between the sequences of the cannabinoid receptors are present in the transmembrane and extracellular domains and may also be involved in the (−)-Δ^9-THC partial agonism/antagonism phenomenon.

According to the above data, (−)-Δ^9-THC does not mediate a strong signal through the CB2 receptor. It has been shown that cannabinoids affect cAMP levels and other modulatory factors in immune system cells, but the concentrations of (−)-Δ^9-THC used in these experiments exceed those required to mediate effects in the central nervous system (19, 20). Moreover, the exact repertoire of cannabinoid binding sites in these cells is not completely clear. Cannabinoids have been shown to modulate proliferation of B-cells, and the CB2 receptor was implicated in this activity; however, (−)-Δ^9-THC was much less potent than CP55,940 and WIN 55,212–2 (18). The exact nature of the signaling process of the cannabinoid receptors in these cells remains to be elucidated. Only with the development of specific potent antagonists for CB2, like the one that has been developed for CB1 (36), will it be possible to observe the effects of blocking the activities of the CB2 receptor in both *in vivo* and *in vitro* systems. The results presented above indicate that (−)-Δ^9-THC is a weak antagonist for the CB2 receptor. Its structure can therefore serve as a model for the chemical synthesis of such an antagonist.

Acknowledgments—We thank Drs. T. Bonner (NIMH, NIH, Bethesda, MD), S. Munro (Cambridge, UK), and T. Pfeuffer (Düsseldorf, Germany) for their kind donations of CB1, CB2, and AC type V cDNA, respectively.

REFERENCES

1. Gerard, C. M., Mollereau, C., Vassart, G. & Parmentier, M. (1991) *Biochem. J.* **279,** 129–134
2. Matsuda, L. A., Lolait, S. J., Browstein, M. J., Young, A. C. & Bonner, T. I. (1990) *Nature* **346,** 561–564
3. Mackie, K. & Hille, B. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 3825–3829
4. Mackie, K., Devane, W. & Hille, B. (1993) *Mol. Pharmacol.* **44,** 498–503
5. Dill, J. & Howlett, A. (1988) *J. Pharmacol. Exp. Ther.* **244,** 1157–1163
6. Bidaut-Russel, M., Devane, W. A. & Howlett, A. C. (1990) *J. Neurochem.* **55,** 21–26
7. Felder, C. C., Veluz, J. S., Williams, H. L., Briley, E. M. & Matsuda, L. A. (1992) *Mol. Pharmacol.* **42,** 313–314
8. Vogel, Z., Barg, J., Levy, R., Saya, D., Heldman, E. & Mechoulam, R. (1993) *J. Neurochem.* **61,** 352–355
9. Compton, D. R., Rice, K. C., De Costa, B. R., Razdan, R. K., Melvin, L. S., Johnson, M. R. & Nartin, B. R. (1993) *J. Pharmacol. Exp. Ther.* **265,** 218–226
10. Lynn, A. & Herkenham, M. (1994) *J. Pharmacol. Exp. Ther.* **268,** 1612–1623
11. Facci, L., Dal Toso, R., Romanello, S., Buriani, A., Skaper, S. D. & Leon, A. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 3376–3380
12. Kaminski, N. E., Abood, M. E., Kessler, F. K., Martin, B. R. & Shatz, A. R. (1992) *Mol. Pharmacol.* **42,** 736–742
13. Munro, S., Thomas, K. L. & Abu-Shaar, M. (1993) *Nature* **365,** 61–65
14. Slipetz, D. M., O'Neil, G. P., Favreau, L., Dufresne, C., Gallant, M., Gareau, Y., Guay, D., Labelle, M. & Metters, K. (1995) *Mol. Pharmacol.* **48,** 352–361
15. Bayewitch, M., Avidor-Reiss, T., Levy, R., Barg, J., Mechoulam, R. & Vogel, Z. (1995) *FEBS Lett.* **375,** 143–147
16. Howlett, A., Johnson, M., Melvin, L. & Milne, G. (1987) *Mol. Pharmacol.* **33,** 297–302
17. Howlett, A., Qualy, J. M. & Khachaturian, L. L. (1986) *Mol. Pharmacol.* **29,** 161–165
18. Derocq, J. M., Ségui, M., Marchand, J., Le Fur, G. & Casellas, P. (1995) *FEBS Lett.* **369,** 177–182
19. Kaminski, N. E., Koh, W., Yang, K., Lee, M. & Kessler, F. (1994) *Biochem. Pharmacol.* **48,** 1899–1908
20. Lee, M., Yang, K. H. & Kaminski, N. E. (1995) *J. Pharmacol. Exp. Ther.* **275,** 529–536
21. López-Cepero, M., Friedman, M., Klein, T. & Friedman, H. (1986) *J. Leukocyte Biol.* **39,** 679–686
22. Felder, C. C., Joyce, K. E., Briley, E. M., Mansouri, J., Mackie, K., Blond, O., Lai, Y., Ma, A. L. & Mitchell, R. L. (1995) *Mol. Pharmacol.* **48,** 443–450
23. Devane, W. A., Breuer, A., Sheskin, T., Järbe, T. U. C., Eisen, M. S. & Mechoulam, R. (1992) *J. Med. Chem.* **35,** 2065–2069
24. Mechoulam, R., Braun, P. & Gaoni, Y. (1972) *J. Am. Chem. Soc.* **94,** 6159–6165
25. Mechoulam, R., Langer, N., Breur, A. & Zahalka, J. (1990) *Tetrahedron: Asymmetry* **1,** 315–319
26. Wallach, J., Droste, M., Kluxen, F. W., Pfeuffer, T. & Frank, R. (1994) *FEBS Lett.* **338,** 257–263
27. Keown, W. A., Campbell, C. R. & Kucherlapati, R. S. (1990) *Methods Enzymol.* **185,** 527–537
28. Levitzki, A. (1984) *Receptors, A Quantitative Approach*, pp. 19–36, The Benjamin/Cummings Publishing Company, Menlo Park, CA
29. Salomon, Y. (1991) *Methods Enzymol.* **195,** 22–28
30. DeLean, A., Munson, P. J. & Rodbard, D. (1988) *A User's Guide to ALLFIT*, pp. 97–102, National Institutes of Health, Bethesda, MD
31. Howlett, A. C. & Fleming, R. M. (1984) *Mol. Pharmacol.* **26,** 532–538
32. Fride, E., Barg, J., Levy, R., Saya, D., Heldman, E., Mechoulam, R. & Vogel, Z. (1995) *J. Pharmacol. Exp. Ther.* **272,** 699–707
33. Savarese, M. & Fraser, M. (1992) *Biochem. J.* **283,** 1–19
34. Heldman, E., Barg, J., Fisher, A., Levy, R., Pittel, Z., Zimlichman, R., Kushnir, M. & Vogel, Z. (1996) *Eur. J. Pharmacol.*, in press
35. Buford, N. T., Tobin, A. B. & Nahorski, S. R. (1995) *J. Pharmacol. Exp. Ther.* **274,** 134–142
36. Rinaldi-Carmona, M., Barth, F., Héaulme, M., Shire, D., Calandra, B., Congy, C., Martinez, S., Maruani, J., Néliat, G., Caput, D., Ferrara, P., Soubrié, P., Brelière, J. C. & Le Fur, G. (1994) *FEBS Lett.* **350,** 240–244