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Short communication

Relative efficacies of cannabinoid CB_1 receptor agonists in the mouse brain

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Abstract

We measured (−)-5-(1,1-dimethylheptyl)-2-[5-hydroxy-2-(3-hydroxypropyl)cyclohexyl]-phenol (CP 55,940)-, (−)11-OH-Δ^8-tetrahydrocannabinol-dimethylheptyl (HU-210)-, anandamide- and Δ^9-tetrahydrocannabinol-stimulated G protein activation in mouse brain using the [^{35}S]GTPγS functional assay. The K_i values for these drugs were determined by agonist competition binding with the cannabinoid CB_1 receptor antagonist [^{3}H]*N*-(piperidin-1-yl-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamidehydrochloride ([^{3}H]SR141716A). This information was used to calculate the efficacy for drug stimulation of G protein activity. The rank order of efficacy was CP 55,940 > HU-210 > anandamide > Δ^9-tetrahydrocannabinol with the latter two drugs being partial agonists. Since efficacy values relate receptor occupancy to functional responses, we believe efficacy values are a better measure of drug-mediated functional responses compared with measurements of drug potency. © 1997 Elsevier Science B.V.

Keywords: Cannabinoid; Cannabinoid receptor; Drug efficacy; Brain, mouse; (Partial agonist); Tetrahydrocannabinol

1. Introduction

Cannabinoid drugs have been demonstrated to exert analgesic, antiemetic and anticonvulsive properties, to reduce body temperature in experimental animals and to decrease intraocular pressure in glaucoma patients (Dewey, 1986; Hollister, 1986). These drugs demonstrate varied potencies in analgesia studies as well as different ED_{50} ratios between therapeutically desirable effects such as analgesia and side effects such as catalepsy and hypothermia (Abood and Martin, 1992). The cloning of the cannabinoid CB_1 receptor from a rat cerebral cortex cDNA library clearly established that many cannabinoid drug effects are receptor mediated (Matsuda et al., 1990). These cannabinoid receptors are, in turn, coupled to pertussis toxin-sensitive G proteins (Mackie and Hille, 1992; Pacheco et al., 1993; Welch et al., 1995). Recently the binding of [^{35}S]GTPγS has been used to determine the activation of G proteins by agonist-stimulated cannabinoid receptors in vitro (Selley et al., 1996). Utilizing [^{35}S]GTPγS binding, we can compare the abilities of different cannabinoid drugs to activate second messenger systems within cells and tissues.

The concept of drug efficacy as a measure of drug activity was first proposed by Stephenson (1956). As proposed, efficacy is a mathematical expression that relates receptor occupancy with a functional response thus should be an accurate description of drug effects in cell systems. Drugs that induce greater functional responses at low receptor occupancies have higher numerical efficacy values. In the results presented below, we determined the EC_{50} values of four different cannabinoid drugs to activate G proteins in mouse brain membranes as measured by the [^{35}S]GTPγS binding assay. We also determined the K_i value of each agonist in competition binding with the radiolabeled cannabinoid CB_1 receptor-specific antagonist [^{3}H] *N*-(piperidin-1-yl-5-(4-chlorophenyl)-1-(2,4-dichloro-

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phenyl)-4-methyl-1H-pyrazole-3-carboxamidehydrochloride ([^{3}H]SR141716A) (Rinaldi-Carmona et al., 1994). Using these data we calculated efficacy values for cannabinoid drugs in the mouse brain as a sensitive measure of drug activity. These methods allow for the in vitro screening of drugs to determine which are most active at cannabinoid CB_1 receptors. This is the first report that quantifies efficacy for cannabinoid drugs.

2. Materials and methods

2.1. Cannabinoid drugs

Δ^9-Tetrahydrocannabinol and anandamide were purchased from Research Biochemicals International (Natick, MA, USA). (−)-5-(1,1-dimethylheptyl)-2-[5-hydroxy-2-(3-hydroxypropyl)cyclohexyl]-phenol (CP 55,940) was the gift of Pfizer Pharmaceuticals (Groton, CT, USA). (−)11-OH-Δ^8-tetrahydrocannabinol-dimethylheptyl (HU-210) was the kind gift of Raphael Mechoulam (Hebrew University, Jerusalem, Israel). The cannabinoid CB_1 receptor antagonist, SR141716A, was the gift of Sanofi Pharmaceuticals (Montpellier, France). Due to the hydrophobicity of cannabinoids, most nonradiolabeled cannabinoids were dissolved at or diluted to a concentration of 2 mM in absolute ethanol and further diluted 1:1 with assay buffer (25 mM Tris–HCl, 150 mM NaCl, 2.5 mM $MgCl_2$, 1 mM EDTA, 0.25% bovine serum albumin, 50 μM GDP, 30 μM bestatin, 10 μM captopril and 0.1 mM phenylmethylsulfonyl fluoride, pH 7.4) to prepare a 1 mM stock solution for use in assays. Anandamide (5 mg/ml in ethanol) was diluted in assay buffer to prepare a 1 mM stock solution. The ethanol concentration did not exceed 0.5% at the highest concentrations of cannabinoids used in these assays.

2.2. Membrane preparation

Male (Institute for Cancer Research, ICR) mice, weighing 20–30 g (Harlan Sprague Dawley, Indianapolis, IN, USA) were killed by cervical dislocation. Whole brains were removed and homogenized (10 strokes with a teflon homogenizer) in TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 7.4). After centrifugation, the membrane pellet was homogenized (8 strokes as above) in assay buffer and incubated (30 min, 30°C) to degrade endogenous ligands. Membranes were then centrifuged, resuspended in assay buffer and used at a final density of $OD_{280} = 0.7$ in both the [^{35}S]GTPγS binding and competition binding assays.

2.3. [^{35}S]GTPγS binding assay

Membranes prepared from mouse brain were incubated with increasing concentrations of each agonist in the presence of 0.1 nM [^{35}S]GTPγS (1000–1500 Ci/mmol, DuPont New England Nuclear, Boston, MA, USA) in a total volume of 1 ml of assay buffer in duplicate. After incubation (90 min, 30°C), the reaction was terminated by rapid filtration under vacuum through Whatman GF/B glass fiber filters, followed by four washes with ice-cold 25 mM Tris/120 mM NaCl, pH 7.4. Filters were pretreated with assay buffer prior to filtration to reduce nonspecific binding. Bound radioactivity was measured by liquid scintillation spectrophotometry after an overnight extraction with EcoLite® (ICN, Biomedicals, Costa Mesa, CA, USA) scintillation cocktail.

2.4. Determination of agonist inhibition of [^{3}H]SR141716A binding

Mouse brain membranes were incubated in duplicate under identical conditions as described above with 1 nM [^{3}H]SR141716A (45 or 52 Ci/mmol, Amersham, Arlington Heights, IL, USA) and increasing concentrations of cannabinoid agonist followed by filtration through Whatman GF/B glass fiber filters. The filters were pretreated in 0.5% polyethylenimine to reduce nonspecific binding. Specific agonist binding was determined as the maximal agonist displacement of [^{3}H]SR141716A. Filter bound radioactivity was determined as above.

2.5. Data analysis

Data from [^{35}S]GTPγS binding and competition binding assays were analyzed using Prism® (GraphPad, San Diego, CA, USA). [^{35}S]GTPγS binding data were fit to a sigmoidal dose–response curve with a Hill slope fixed to 1.0 to determine the EC_{50} value of cannabinoid-stimulated binding as well as the maximal response. Data from the competition binding of [^{3}H]SR141716A with cannabinoid drugs were analyzed by a one-site competition model to determine the IC_{50} value. The K_i values of cannabinoid drugs were determined from the IC_{50} using the Cheng and Prusoff equation ($K_i = IC_{50}/(1 + [\text{ligand}]/K_d)$) (Cheng and Prusoff, 1973). The mean K_d value for [^{3}H]SR141716A binding in this buffer system is 10.4 nM ($n = 3$, data not shown). K_i and EC_{50} values and maximal [^{35}S]GTPγS binding were calculated for each experiment and then averaged. Differences between cannabinoid drugs in stimulation of [^{35}S]GTPγS binding over basal levels were examined for statistically significant differences using analysis of variance and the Newman–Keuls multiple range test.

2.6. Efficacy calculations

Cannabinoid drug efficacy was calculated using the following equation:

$$\text{efficacy} = E_{\text{max-A}}/E_{\text{max}} \times (K_d/EC_{50} + 1) \times \frac{1}{2}$$

where $E_{max\text{-}A}$ = the maximum response given by a particular drug and E_{max} = the maximum agonist-stimulated response in the test system (Ehlert, 1985). Relative efficacy was determined by dividing the efficacy value of a drug by the efficacy value calculated for CP 55,940. The K_d was determined as the K_i in these experiments.

3. Results

Binding of CP 55,940, HU-210, anandamide and Δ^9-tetrahydrocannabinol in the presence of [^{3}H]SR141716A (1 nM) to mouse brain membranes was determined (Table 1). The K_i values of these drugs were 126, 1.6, 585 and 144 nM, respectively. We also calculated the EC_{50} of [^{35}S]GTPγS binding to mouse brain membranes as a measure of cannabinoid drug-mediated G protein activation under the same buffer conditions as in the competition binding assays. The EC_{50} values for CP 55,940 and HU-210 and anandamide were 61.7, 2.3 and 846 nM, respectively. We have previously reported [^{35}S]GTPγS binding data for Δ^9-tetrahydrocannabinol (Burkey et al., 1997). When these data were analyzed by a fixed slope sigmoidal dose–response curve an EC_{50} of 70.9 nM was obtained. CP 55,940, HU-210, anandamide and Δ^9-tetrahydrocannabinol stimulated maximal [^{35}S]GTPγS binding of 136, 123, 96 and 37% over basal levels, respectively. Cannabinoid (1 μM)-stimulated [^{35}S]GTPγS binding was blocked by SR141716A (10 μM) indicating that G protein activation was mediated through cannabinoid CB_1 receptors for all drugs tested (data not shown).

The K_i and EC_{50} values and the percent increase over basal [^{35}S]GTPγS binding values were used to calculate efficacy (Table 1)(Ehlert, 1985). We defined CP 55,940 as a full agonist in this system as this drug stimulated the greatest increase in [^{35}S]GTPγS binding, thus maximal CP 55,940-stimulated binding was defined as E_{max}. The order of efficacy from most to least efficacious cannabinoid drug in the mouse brain is CP 55,940 > HU-210 > anandamide > Δ^9-tetrahydrocannabinol.

We replotted the data from Table 1 as the percent maximal functional response versus percent receptor occupancy by drug (Fig. 1). These data suggest that there are few spare receptors for cannabinoid drugs in the mouse brain as both CP 55,940 and HU-210 must bind nearly 100% of the receptors present to obtain maximal activation of G proteins. Conversely, anandamide and Δ^9-tetrahydrocannabinol did not stimulate maximal [^{35}S]GTPγS binding at receptor saturation consistent with the interpretation that these drugs are partial agonists.

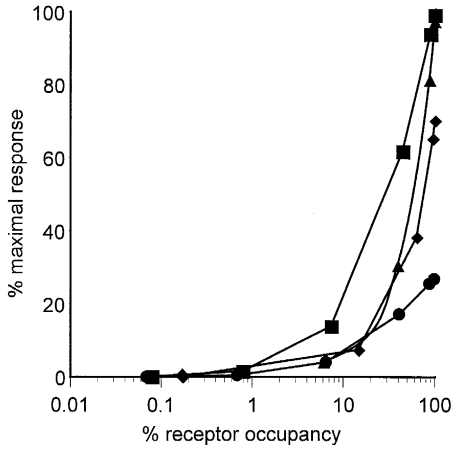

Fig. 1. Percent maximal [^{35}S]GTPγS binding versus % receptor occupancy. Percent maximal [^{35}S]GTPγS binding and receptor occupancy were calculated at several agonist concentrations from data presented in Table 1 and replotted for CP 55,940 (■), HU-210 (▲), anandamide (♦) and Δ^9-tetrahydrocannabinol (●). Both CP 55,940 and HU-210 must occupy nearly all available receptors to stimulate maximal [^{35}S]GTPγS binding. Anandamide and Δ^9-tetrahydrocannabinol did not stimulate maximal [^{35}S]GTPγS binding even at receptor saturation.

4. Discussion

This report represents the first quantitative determination of efficacy values for cannabinoid drugs. We observed that the rank order of efficacy (CP 55,940 > HU-210 > anandamide > Δ^9-tetrahydrocannabinol) does not correspond to the rank order of potency (HU-210 > CP 55,940 ≈ Δ^9-tetrahydrocannabinol ≫ anandamide). This difference in rank order reflects the fact that efficacy measures the relationship between receptor occupancy and activation of functional responses whereas potency measurements only examine the functional response. We believe that efficacy represents a superior pharmacodynamic measure

Table 1
Efficacies of cannabinoid receptor agonists in mouse brain membranes

	K_i (nM)	EC_{50} (nM)	% Stimulation	*n*	Efficacy	Relative efficacy
CP 55,940	126 ± 73	61.7 ± 11.6	136 ± 11	3	1.52	1.00
HU-210	1.56 ± 0.25	2.26 ± 0.38	123 ± 4	4	0.77	0.51
Anandamide	585 ± 105	846 ± 84	96 ± 5	3	0.60	0.39
Δ^9-Tetra-hydrocannabinol	144 ± 63	70.9 ± 31.7	37 ± 2	3	0.41	0.27

K_i values were determined from [^{3}H]SR141716A competition binding experiments and EC_{50} values from [^{35}S]GTPγS binding experiments. % stimulation represents maximal stimulation of [^{35}S]GTPγS binding over basal as calculated by Prism®. K_i, EC_{50} and % stimulation values represent the mean ± S.E.M.

as efficacy calculations allow one to determine the contribution of both drug affinity for receptor and receptor coupling efficiency to drug potency. Additionally, efficacy calculations quantify the partial agonist nature of some drugs whereas this information is not included in potency determinations. Thus, even though HU-210 is more potent than CP 55,940 in the [^{35}S]GTPγS binding assay, for any level of receptor occupancy CP 55,940 stimulates a slightly greater functional response. Additionally, the efficacy calculations support previous findings that anandamide and Δ^9-tetrahydrocannabinol are partial agonists (Mackie et al., 1993; Sim et al., 1996; Burkey et al., 1997).

The mechanism responsible for the variability in efficacy of cannabinoid drugs in the mouse brain is unclear. Competition binding experiments indicate that these agonists bind comparable numbers of cannabinoid receptors with agonist inhibition of [^{3}H]SR141716A binding curves in agreement with the law of mass action (data not shown). These findings exclude an inability of less efficacious drugs to bind the receptor as a reason for low efficacy. One possible explanation for partial agonist activity could involve cannabinoid CB_1 receptor subtypes. As cannabinoid receptors are incompletely characterized, it is possible that multiple variants of cannabinoid receptor exist in the brain that demonstrate identical ligand binding characteristics. If a drug bound to multiple cannabinoid CB_1 receptor subtypes but only activated one of them, it would demonstrate low efficacy as compared to agonists that bound and activated all subtypes. An alternate explanation is that HU-210-, anandamide- and Δ^9-tetrahydrocannabinol-bound cannabinoid receptors do not activate G proteins as efficiently as receptors bound to CP 55,940. Cannabinoid CB_1 receptors bound to drugs other than CP 55,940 may either recognize fewer subtypes of G protein (e.g., $G_{i\alpha1}$, $G_{i\alpha2}$, G_o, etc.) as compared to CP 55,940-bound receptor or may be less efficient at stimulating the same pool of G proteins. Further research will be necessary to elucidate which of these mechanisms is correct.

In this report we utilize the results of cannabinoid CB_1 receptor binding and [^{35}S]GTPγS binding assays to determine the efficacy of four cannabinoid drugs to activate cannabinoid CB_1 receptor-coupled G proteins in membranes isolated from mouse brain. This assay provides an in vitro screening method to test the efficacy of cannabinoid drugs. Efficacy values should provide a more sensitive measure of the ability of cannabinoid drugs to stimulate second messenger systems as compared to drug potency determinations.

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References

Abood, M.E., Martin, B.R., 1992. Neurobiology of marijuana abuse. Trends Pharmacol. Sci. 13, 201–206.

Burkey, T.H., Quock, R.M., Consroe, P., Roeske, W.R., Yamamura, H.I., 1997. Δ^9-tetrahydrocannabinol is a partial agonist of cannabinoid receptors in mouse brain. Eur. J. Pharmacol. 323, R3–R4.

Cheng, Y., Prusoff, W.H., 1973. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50% inhibition (IC_{50}) of an enzymatic reaction. Biochem. Pharmacol. 22, 3099–3108.

Dewey, W., 1986. Cannabinoid pharmacology. Pharmacol. Rev. 38, 151–178.

Ehlert, F.J., 1985. The relationship between muscarinic receptor occupancy and adenylate cyclase inhibition in the rabbit myocardium. Mol. Pharmacol. 28, 410–421.

Hollister, L.E., 1986. Health aspects of cannabis. Pharmacol. Rev. 38, 1–20.

Mackie, K., Hille, B., 1992. Cannabinoids inhibit N-type calcium channels in neuroblastoma–glioma cells. Proc. Natl. Acad. Sci. USA 89, 3825–3829.

Mackie, K., Devane, W.A., Hille, B., 1993. Anandamide, an endogenous cannabinoid, inhibits calcium currents as a partial agonist in N18 neuroblastoma cells. Mol. Pharmacol. 44, 498–503.

Matsuda, L.A., Lolait, S.J., Brownstein, M.J., Young, A.C., Bonner, T.I., 1990. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346, 561–564.

Pacheco, M.A., Ward, S.J., Childers, S.R., 1993. Identification of cannabinoid receptors in cultures of rat cerebellar granule cells. Brain Res. 603, 102–110.

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., LeFur, G., 1994. SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. FEBS Lett. 350, 240–244.

Selley, D.E., Stark, S., Sim, L.J., Childers, S.R., 1996. Cannabinoid receptor stimulation of guanosine-5′-O-(3-[^{35}S]thio)triphosphate binding in rat brain membranes. Life Sci. 59, 659–668.

Sim, L.J., Hampson, R.E., Deadwyler, S.A., Childers, S.R., 1996. Effects of chronic treatment with Δ^9-tetrahydrocannabinol on cannabinoid-stimulated [^{35}S]GTPγS autoradiography in rat brain. J. Neurosci. 16, 8057–8066.

Stephenson, R.P., 1956. A modification of receptor theory. Br. J. Pharmacol. 11, 379–393.

Welch, S.P., Thomas, C., Patrick, G.S., 1995. Modulation of cannabinoid-induces antinociception after intracerebroventricular versus intrathecal administration to mice: Possible mechanisms for interaction with morphine. J. Pharmacol. Exp. Ther. 272, 310–321.