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Cannabinoid agonists and antagonists discriminated by receptor binding in rat cerebellum

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1 The effect of allosteric regulators on the binding affinity of a number of cannabinoid receptor ligands of varying efficacy in the rat cerebellum was investigated.

2 Radioligand ([^{3}H]-SR141716A) competition curves were constructed in the presence or absence of sodium ions, magnesium ions and guanine nucleotides.

3 It was found that the presence of these allosteric regulators did not affect the affinity of the two antagonists used but did cause a significant decrease in the affinity of full and partial agonists.

4 This reduction in affinity ranged from a 3.67 fold rightward shift of the displacement curve of a mixed agonist/antagonist (3-(6-cyano-2-hexynyl)-delta-8-tetrahydrocannabinol-O-823) to a 38 fold rightward shift for 3-(1,1-dimethyl-6-dimethylcarboxamide)-delta-8-tetrahydrocannabinol (O-1125), a full agonist.

5 In summary, the results of this study suggest a simple method for the inference of functional data using the classical radioligand binding assay.

Keywords: Radioligand binding; [^{3}H]-SR141716A; CB_1 receptors; cannabinoids; efficacy; rat cerebellum; allosteric regulation

Abbreviations: BSA, fatty acid free bovine serum albumin; CB_1, central cannabinoid receptor; CB_2, peripheral cannabinoid receptor; CP 55,940, (−)-3-[2-hydroxyl-4-(1,1-dimethylheptyl)-phenyl]-4-[3 hydroxypropyl] cyclohexan-1-ol; GTPγS, guanosine 5′-O-(3-[^{35}S]-thio)-triphosphate; HU-210, (−)-11-hydroxy-delta-8-tetrahydrocannabinol-1,1-dimethylheptyl; O-689, 2-methylarachidonyl-(2′-fluoroethyl)amide; O-806, 3-(6-bromo-2-hexynyl)-Δ^8-THC; O-823, 3-(6-cyano-2-hexynyl)-Δ^8-THC; O-1125, 3-(1,1-dimethyl-6-dimethylcarboxamide)-Δ^8-THC; O-1238, 3-(6-Azido-2-hexenyl)-Δ^8-THC; O-1302, N-(piperidin-1-yl)-1-(2,4,dichlorophenyl)-4-methyl-5-(4 pentylphenyl)-1H-pyraxole-3-carboxamide; SR141716A, N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamidehydrochloride; THC, tetrahydrocannabinol; WIN 55212-2, (R)-(+)-[2,3-dihydro-5-methyl-3-[(4-morpholinyl)methyl]pyrolo[1,2,3-*de*]-1,4-benzoxazin-6-yl](1-naphthalenyl)methanone

Introduction

Since the isolation of delta-9-tetrahydrocannabinol as the principal psychoactive constituent of marijuana (Gaoni & Mechoulam, 1964), the field of cannabinoid pharmacology has developed from the study of psychoactive drugs to the characterization of an endogenous system. The principal findings to date include the discovery of two specific G-protein coupled receptors (termed CB_1 and CB_2) and the isolation of several endogenous ligands for these receptors, the best characterized of which is an ethanolamide derivative of arachidonic acid known as anandamide (Matsuda *et al*., 1990; Munro *et al*., 1993; Devane *et al*., 1992). The synthesis of numerous receptor ligands and the development of suitable assays with which to investigate both these ligands and the receptors themselves has been integral to our increased understanding of this field.

The radioligand binding assay has been used extensively to assess the affinity of ligands at CB_1 and CB_2 receptors (Devane *et al*., 1988; Showalter *et al*., 1996). However, the major drawback of the binding assay is an inability to confer functional information regarding the ligand, whether it is an agonist or an antagonist. It has long been established for many G-protein coupled receptors that the binding of agonists may be decreased by allosteric regulators such as sodium ions and guanine nucleotides in accordance with the two-state model of receptor binding, and similarly, that antagonists are not affected by such regulation (Devane *et al*., 1988; Pert *et al*., 1973; Childers & Snyder, 1980). In 1994, Rinaldi-Carmona *et al*. (1994) announced the development of SR141716A, a high affinity CB_1-selective cannabinoid antagonist. The subsequent radiolabelling of this compound has allowed radioligand binding assays using a labelled antagonist rather than the labelled agonists which had previously been used, examples of which are [^{3}H]-CP 55,950, [^{3}H]-WIN 55212-2 and [^{3}H]-HU-243 (Devane *et al*., 1988, 1992; Kuster *et al*., 1993). A recent report by Houston & Howlett (1998) used the tritiated form of SR141716A to extensively investigate the effects of sodium ions and a non-hydrolyzable guanine nucleotide on the binding of two cannabinoid receptor agonists, desacetyllevonantradol and WIN 55212-2. Other extensive studies have also been carried out using this compound and several subtle differences between agonist and antagonist cannabinoid receptor binding have been observed (Thomas *et al*., 1998). However, the development of this compound also presents the possibility of a novel type of cannabinoid binding assay. As previously stated, the ability of certain ions and guanine nucleotides to decrease the affinity of efficacious receptor ligands whilst leaving antagonists unaffected may now be exploited by the use of a radioligand that should itself be affected by these allosteric modulators of binding.

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Our laboratory has previously presented data regarding the efficacies of many cannabinoid receptor ligands in the GTPγS binding assay using rat cerebellar membranes (Griffin *et al*., 1998, 1999), known to contain a very high concentration of CB_1 receptors (Herkenham *et al*., 1991). In this study, we have investigated the possibility of a relationship between a compounds efficacy and the influence of allosteric regulators on its binding to cannabinoid receptors in order to determine whether such a method may be applied as a first-step functional assay for cannabinoid receptor ligands.

Methods

Cerebella from male Sprague-Dawley rats were extracted on ice and pooled. Membrane homogenates were then prepared as previously described (Griffin *et al*., 1998), with the exception that a sodium-free buffer (50 mM Tris HCl, 3 mM $MgCl_2$, 1 mM EDTA, pH 7.4) was used throughout the homogenization and freezing process.

The methods used for radioligand binding were essentially those described by Compton *et al*. (1993) with minor exceptions. Binding was initiated by the addition of 20 μg membrane protein to siliconized tubes containing 0.35 nM [^{3}H]-SR141716A, the competing ligand and a sufficient volume of buffer A (mM): Tris HCl 50, $MgCl_2$ 3, EDTA 1, 0.1 % BSA, pH 7.4); buffer B (mM) Tris HCl 50, $MgCl_2$ 9, EDTA 1, NaCl 150, GDP 100, GTPγS 0.05, 0.1% BSA, pH 7.4, or buffer A including one (mM) of NaCl 150, $MgCl_2$ 9, GDP 100, GTPγS 0.05 to bring the total volume to 0.5 ml. The composition of buffer B was designed to exactly duplicate the conditions used for the GTPγS binding experiments on which most of the efficacy information is based. Total binding of [^{3}H]-SR141716A to rat cerebellar membranes was linear at protein concentrations from 10 to 80 μg 0.5 ml^{-1}. Specific binding reached a plateau above 30 μg 0.5 ml^{-1}. Therefore, 20 μg 0.5 ml of rat cerebellar membrane was used in all assays. The addition of 1 μM SR141716A was used to assess non-specific binding.

Materials

Male Sprague-Dawley rats (150–250 g) were obtained from Harlan (Dublin, VA, U.S.A.). GDP and GTPγS were purchased from Boehringer Mannheim (Indianapolis, IN, U.S.A.). [^{3}H]-SR141716A (55 Ci $mmol^{-1}$) was purchased from Amersham (Arlington Heights, IL, U.S.A.). Other reagent grade chemicals were obtained from Sigma (St. Louis, MO, U.S.A.). Δ^9-tetrahydrocannabinol (Δ^9-THC) was obtained from the National Institute on Drug Abuse. CP 55,940, CP 55,244 and SR 141716A were provided by Pfizer Inc. (Groton, CT, U.S.A.), HU-210 was provided by Prof Raphael Mechoulam (Hebrew University, Jerusalem, Israel) and WIN 55212-2 was purchased from Research Biochemicals International (Natick, MA, U.S.A.). Anandamide, O-689 (2-methylarachidonyl-(2′-fluoroethyl) amide), O-806 (3-(6-bromo-2-hexynyl)-Δ^8-THC), O-823 (3-(6-cyano-2-hexynyl)-Δ^8-THC), O-1125 (3-(1,1-dimethyl-6-dimethylcarboxamide)-delta-8-tetrahydrocannabinol), O-1238 (3-(6-Azido-2-hexenyl)-Δ^8-THC) and O-1302 (N-(piperidin-1-yl)-1-(2,4,dichlorophenyl)-4-methyl-5-(4-pentylphenyl)-1H-pyraxole-3-carboxamide) were synthesized by Dr Raj Razdan (Organix, Inc., Woburn, MA, U.S.A.). All compounds were stored as 1 mg ml^{-1} solutions in ethanol at −20°C.

Data analysis

Data are reported as means ± s.e.means of three to eight experiments, each performed in triplicate. B_{max} and K_d values obtained from Scatchard analysis of saturation binding curves were determined by the KELL package of binding analysis programs for the Macintosh computer (Biosoft, Milltown, NJ, U.S.A.). Displacement IC_{50} values were determined originally by unweighted least-squares non-linear regression of log concentration-percentage of displacement data and then converted to K_i values using the method of Cheng & Prusoff (1973). Statistical comparisons of K_i values were achieved using unpaired two-tailed Students *t*-test ($P<0.05$).

Results

Saturable, high affinity binding was obtained with [^{3}H]-SR141716A in rat cerebellar membranes, compatible with a single site (Hill coefficient = 1.07 (buffer A) 1.09 (buffer B)). The saturation experiments were conducted with [^{3}H]-SR141716A concentrations of 0.1–5 nM, and the K_d value was calculated to be 0.36 ± 0.05 nM and the B_{max} to be 4.39 ± 0.47 pmol mg^{-1} protein using buffer A and 0.35 ± 0.10 nM and 4.80 ± 0.25 pmol mg protein using buffer B. The [^{3}H]-SR141716A K_d and the B_{max} values were not significantly affected by the change of buffer (Unpaired Students *t*-test, two-tailed, $P<0.05$). The compounds used for this study were selected for their range of efficacies, based predominantly on their activities in the GTPγS binding assay in rat cerebellar membranes. We have previously described SR141716A as a cannabinoid receptor antagonist in this assay (Griffin *et al*., 1998). O-1302, a structural derivative of SR141716A, also acts as a competitive cannabinoid receptor antagonist, antagonising WIN 55212-2- and CP 55,940-stimulated GTPγS binding with a K_B value of 0.23 (0.07–0.79) nM (Data not shown). It is also devoid of agonist activity *in vivo* (B.R. Martin, unpublished results). O-806 and O-823 also antagonize cannabinoid receptor agonists in the GTPγS binding assay, but O-823 has previously been described as a very low efficacy partial agonist and both are moderately active *in vivo* (Griffin *et al*., 1999; Pertwee *et al*., 1996; B.R. Martin, unpublished results), hence their classification as mixed agonists/ antagonists at the CB_1 receptor. THC and anandamide stimulate GTPγS binding with low efficacy, whereas O-689, CP 55,940 and O-1238 stimulate GTPγS binding to a significantly greater level, hence their listings as medium efficacy partial agonists. Finally, we have previously described CP 55,244, WIN 55212-2, HU-210 and O-1125 as full agonists in the GTPγS binding assay (Griffin *et al*., 1998, 1999). The structure of each of these compounds is shown in Figure 1.

In order to compare the affinities of ligands in the presence or absence of various allosteric modulators of binding, two buffers were used. Buffer A was a standard assay buffer used for binding experiments, containing solely Tris HCl, EDTA and a low concentration of magnesium ions whereas buffer B was identical to that used for the GTPγS binding experiments, containing GDP, GTPγS, sodium ions and an increased concentration of magnesium ions as well as Tris HCl and EDTA. The initial experiments conducted used a full agonist, WIN 55212-2, and compared its affinity in either buffer A, buffer B or in buffer A containing one of the additional moieties found in buffer B in order to assess the individual contributions to any overall effect which may be seen (Figure 2). Table 1 shows the K_i values of WIN 55212-2 calculated

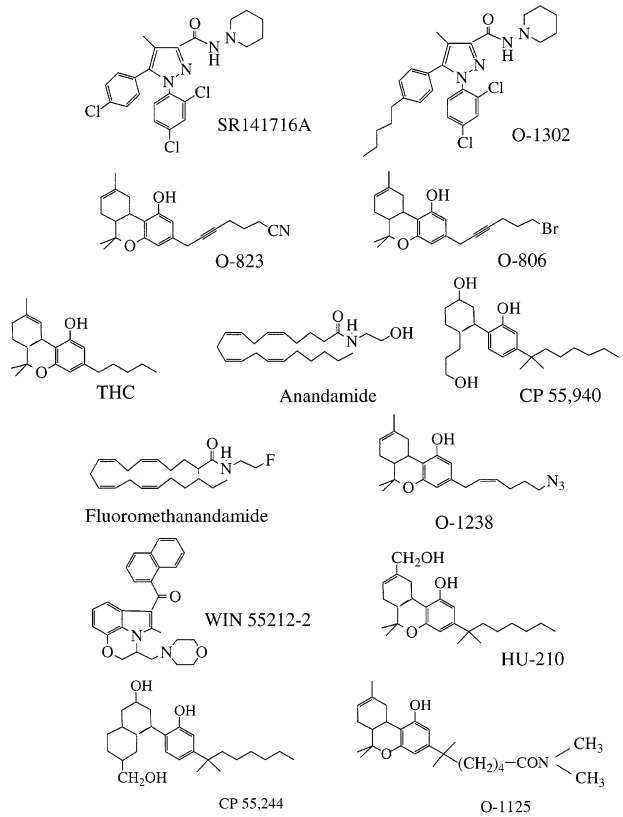

Figure 1 Structure of compounds used in this study.

Table 1 Effects of varying the buffer composition on the K_i value of WIN 55212-2 in rat cerebellar membranes

Buffer used	*WIN 55212-2 K_i*
Buffer A	11.1±3.77
Buffer A+150 mM NaCl	16.9±4.53
Buffer A+9 mM $MgCl_2$	13.9±1.18
Buffer A+100 μM GDP	329±59.0
Buffer A+0.05 nM GTPγS	166±52.0
Buffer B	400±143

Buffer A (mM): Tris HCl, 50; $MgCl_2$, 3; EDTA, 1; 0.1% BSA, pH 7.4; Buffer B: Tris HCl, 50 mM; $MgCl_2$, 9 mM; EDTA, 1 mM; NaCl, 150 mM; GDP, 100 μM; GTPγS 0.05 nM; 0.1% BSA, pH 7.4. All K_i values are expressed in nM as the means±s.e.means of 3–6 experiments each performed in triplicate.

under each of these conditions. The presence of sodium ions and magnesium ions both caused a non-significant reduction in the observed affinity of WIN 55212-2 (unpaired two-tailed Students *t*-test ($P<0.05$)). The guanine nucleotides GDP and GTPγS, however, both caused a significant reduction in the affinity of WIN 55212-2, as did buffer B which contained all of the individual factors. In every case, the Hill slopes were not affected.

Having shown the ability of buffer B to cause a 36 fold reduction in the affinity of WIN 55212-2, a full agonist, the affinity of a range of other compounds of mixed efficacies were then tested in either buffer A or buffer B. The results from these experiments are shown in Table 2. Figure 3 shows the dissociation curves obtained with an antagonist, SR141716A (Figure 3A), a low efficacy partial agonist, THC (Figure 3B), a medium efficacy partial agonist, CP 55,940 (Figure 3C) and a full agonist, O-1125 (Figure 3D). It was found that buffer B

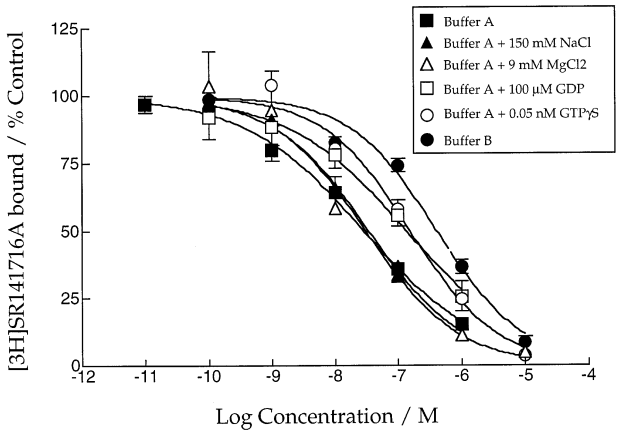

Figure 2 Effect of different experimental conditions on the ability of WIN 55212-2 to displace [^{3}H]-SR141716A in rat cerebellar membranes. The data are expressed as percentage displacement of specific binding; 0.35 nM [^{3}H]-SR141716A was the concentration of radioligand used. Non-specific binding was measured in the presence of 1 μM SR141716A. Data points are the means±s.e.mean of 3–7 experiments performed in triplicate.

caused significant reductions in the affinity of all compounds tested except SR141716A and O-1302, the two antagonists. There was also a trend observed whereby the greater the efficacy of the agonist, the greater the rightward shift of the displacement curve. This observation was not universal with the compounds used in this study, the notable exception being CP 55,244. CP 55,244 acts as a full agonist in the GTPγS binding assay but was markedly less affected by the various allosteric modulators than the other full agonists used.

Discussion

The results presented in this report confirm the ability of various allosteric regulators of binding to directly affect the affinity of cannabinoid receptor agonists, confirming previous observations (Devane *et al*., 1988; Kuster *et al*., 1993; Pacheco *et al*., 1994). Interestingly, sodium and magnesium ions did not have as great an effect on agonist affinity in this study compared with these previous studies. This may be due largely to the different experimental conditions used in this study, and the difference in the experiment conducted. The previous studies measured differences in specific binding of the radiolabelled agonist, WIN 55212-2, as opposed to displacement of a radiolabelled antagonist by WIN 55212-2. In contrast, the guanine nucleotides GDP and GTPγS had a much greater effect. The affinities of the two antagonists used, SR141716A and O-1302, were unaffected by the presence of sodium or magnesium ions or guanine nucleotides. From the results of this study, therefore, it would appear that manipulation of the assay conditions used for the radioligand binding assay may be used as a predictive measure of cannabinoid receptor ligand efficacy.

Although there does not appear to be a direct correlation between the degree of the rightward shift of a ligand's displacement curve and its efficacy, there is a general trend observed with the lowest efficacy compounds affected the least and the highest efficacy compounds the most. This would be consistent with the two-state model of receptor activation. Assuming that higher efficacy, full agonists bind preferentially to the active state of the receptor, any experimental conditions reducing the number of active receptors (buffer B) will likely

Table 2 Effect of varying the buffer composition on the K_i values of cannabinoid receptor ligands in rat cerebellum

Classification	*Compound*	*E_{max} (% stimulation of GTPgS binding)*	K_i/nM *(Buffer A)*	K_i/nM *(Buffer B)*	K_i *ratio*
Antagonist	SR141716A	N/A	0.31±0.08	0.25±0.05	0.81
	O-1302	N/A	0.18±0.03	0.22±0.03	1.22
Mixed agonist/antagonist	O-806	N/A	0.90±0.08	3.30±0.13	3.67**
	O-823	N/A	0.66±0.41	3.74±1.20	5.67*
Low efficacy partial agonist	THC	51 (46–571)[1,a]	68.7±4.68	463±102	6.72*
	Anandamide	45 (32–58)[2,c]	170±36.6	1620±252	9.53*
Medium efficacy partial agonist	O-1238	58 (44–73)[b]	4.63±0.61	34.06±2.23	7.35**
	O-689	97 (55–140)[a]	48.9±13.2	571±178	11.67*
	CP 55,940	114 (97–131)	4.81±1.06	47.4±9.61	9.52**
High efficacy full agonist	HU-210	140 (117–152)[a]	0.37±0.12	5.61±3.12	15.16*
	O-1125	165 (150–181)[b]	1.16±0.12	44.1±7.55	37.98**
	WIN 55212-2	156 (144–169)	11.1±3.77	400±143	36.13*
	CP 55,244	165 (148–183)	0.21±0.04	1.35±0.11	6.43**

*$P<0.05$ (unpaired Student's *t*-test, two-tailed); **$P<0.01$ (unpaired Student's *t*-test, two-tailed). [a]Griffin *et al.*, 1998; [b]Griffin *et al.* 1999; [c]Unpublished data; [1]10 μM GDP; [2]10 μM GDP, No sodium.

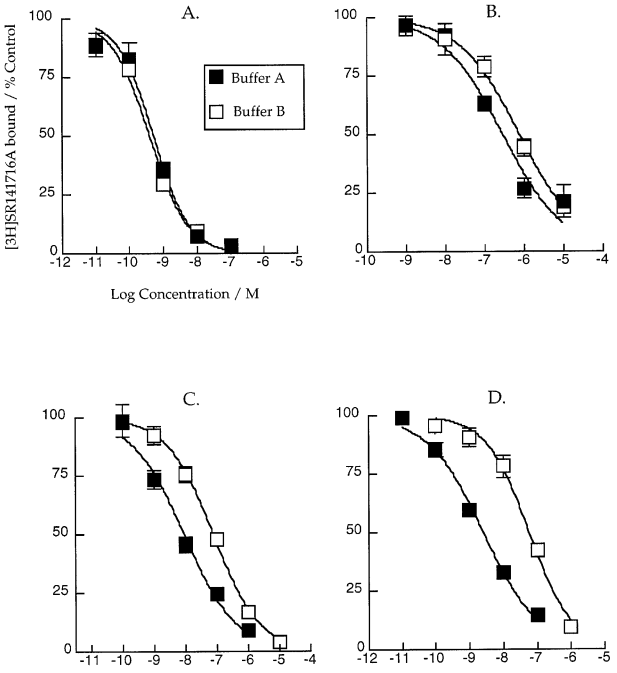

Figure 3 Displacement of bound [^{3}H]-SR141716A from rat cerebellar membranes by SR141716A (A), THC (B), CP 55,940 (C) and O-1125 (D) in the presence of buffer A or buffer B. The data are expressed as percentage displacement of specific binding; 0.35 nM [^{3}H]-SR141716A was the concentration of radioligand used. Non-specific binding was measured in the presence of 1 μM SR141716A. Data points are the means±s.e.mean of 4–8 experiments performed in triplicate.

have a greater effect on the affinity of these compounds than those whose binding may be more evenly distributed between different states of the receptor. The evaluation of further compounds using this methodology may more fully explore the possibility of a direct relationship between efficacy and the extent of allosteric regulation of binding.

The major exception to this observation is CP 55,244, a full agonist in several functional assay systems, which is affected by these allosteric regulators markedly less than the other full agonists used. Why this should be is presently unclear. Interestingly, the other bicyclic compound examined in this series, CP 55,940, also showed somewhat less of an affinity reduction than other agonists of comparable efficacies. This may reflect significant differences in the way by which these ligands bind to the receptor and/or the means by which they induce the conformational change required to activate the receptor. However, this is speculative and further experiments with these compounds are required to satisfactorily explain this difference.

Our previous work with cannabinoid ligands has suggested that the range of efficacies of these compounds is vast, ranging from antagonists through to full agonists. However, using the GTPγS binding assay, several compounds have acted as competitive receptor antagonists in this assay whereas these compounds have clearly been demonstrated to possess efficacy and act as partial agonists, an example being the previously discussed O-823, in other assays. Our previous explanation for this finding related to the very low efficacy of such compounds, and an inability to detect this in the GTPγS assay even when using conditions favoring lower efficacy (for example, by decreasing the GDP concentration and thus increasing the number of receptors in the active state) (Griffin *et al.*, 1998). The results of this study further support this possibility, with the very low degree of rightward shift of the displacement curve of O-823. If O-823 was acting as a true antagonist, then it may be anticipated that the use of the two different assay buffers in these experiments should not affect the affinity of this compound. However, the small shift of the displacement curve does suggest a preference of binding to the active form of the receptor, as for an agonist, and therefore the lack of a detectable agonist effect. Similarly the appearance of a competitive antagonist effect in the GTPγS binding assay may reflect action as a silent ligand. In another tissue, however, which may be relatively richer in active receptors, a significant degree of agonism may then be seen.

There has been debate recently as to whether SR141716A acts as a neutral antagonist, or as an inverse agonist, at the CB_1 receptor. Our results in this study, both in the saturation experiments and the displacement studies show that the various allosteric regulators of binding used in this study do not affect the binding of this compound. Were SR141716A acting as an inverse agonist, it may have been anticipated that its affinity would be increased in the presence of buffer B, with a greater proportion of receptors in the inactive state and thus favoring the binding of a inverse agonist (Leff, 1995).

However, this was not seen and would support the hypothesis that SR141716A is a neutral antagonist under the conditions used in these experiments.

In summary, the results of this study suggest a possible basis for which the radioligand binding assay may be used as an initial step in the assessment of efficacy of a cannabinoid receptor ligand, allowing the delineation of antagonists (such as SR141716A) and partial agonists (such as THC or anandamide) or full agonists (such as WIN 55212-2). With the development of a radiolabelled, high affinity antagonist for the CB_2 receptor (for example, SR144528 (Rinaldi-Carmona *et al*., 1998)) it may also be possible to apply this method to tissues or cells containing CB_2 receptors thus providing a much needed simple functional assay for CB_2 selective ligands. Moreover, these studies indicate that the pharmacological activity of cannabinoids may well be dictated by the receptor environment, this may become particularly pertinent from a viewpoint of clinical applications for these compounds should further receptor subtypes fail to be discovered. Presently, the isolation of particular central pharmacological effects is impossible due to the single receptor subtype involved in mediating cannabinoid effects in the central nervous system, but by using low efficacy compounds, it may become possible to isolate specific effects. Similar studies with brain regions other than the cerebellum would therefore be of great interest to establish how they vary in their receptor environment, and thus how low efficacy agonists compare in their pharmacological activities.

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References

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

CHILDERS, S.R. & SNYDER, S.H. (1980). Differential regulation by guanine nucleotides of opiate agonist and antagonist receptor interactions. *J. Neurochem.,* **34,** 583–593.

COMPTON, D.R., RICE, K.C., DE COSTA, B.R., RAZDAN, R.K., MELVIN, L.S., JOHNSON, M.R. & MARTIN, B.R. (1993). Cannabinoid structure-activity relationships: correlation of receptor binding and in vivo activities. *J. Pharmacol. Exp. Ther.,* **265,** 218–226.

DEVANE, W.A., DYSARZ III, F.A., JOHNSON, M.R., MELVIN, L.S. & HOWLETT, A.C. (1988). Determination and characterization of a cannabinoid receptor in rat brain. *Mol. Pharm.,* **34,** 605–613.

DEVANE, W.A., HANUS, L., BREUER, A., PERTWEE, R.G., STEVENSON, L.A., GRIFFIN, G., GIBSON, D., MANDELBAUM, A., ETINGER, A. & MECHOULAM, R. (1992). Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science,* **258,** 1946–1949.

GAONI, Y. & MECHOULAM, R. (1964). Isolation, structure and partial synthesis of an active constituent of hashish. *J. Amer. Chem. Soc.,* **86,** 1646–1647.

GRIFFIN, G., ATKINSON, P.J., SHOWALTER, V.M., MARTIN, B.R. & ABOOD, M.E. (1998). Evaluation of cannabinoid receptor agonists and antagonists using the [^{35}S]GTPγS binding assay in rat cerebellar membranes. *J. Pharmacol. Exp. Ther.,* **285,** 553–560.

GRIFFIN, G., WRAY, E.J., RORRER, W.K., CROCKER, P.J., RYAN, W.R., SAHA, B., RAZDAN, R.K., MARTIN, B.M. & ABOOD, M.E. (1999). An investigation into the structural determinants of cannabinoid receptor efficacy. *Br. J. Pharmacol.,* **126,** 1575–1584.

HERKENHAM, M., LYNN, A.B., JOHNSON, M.R., MELVIN, L.S., DE COSTA, B.R. & RICE, K.C. (1991). Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autographic study. *J. Neurosci.,* **11,** 563–583.

HOUSTON, D.B. & HOWLETT, A.C. (1998). Differential receptor-G-protein coupling evoked by dissimilar cannabinoid receptor agonists. *Cell Signal.,* **10,** 667–674.

KUSTER, J.E., STEVENSON, J.I., WARD, S.J., D'AMBRA, T.E. & HAYCOCK, D.A. (1993). Aminoalkylindole binding in rat cerebellum: selective displacement by natural and synthetic cannabinoids. *J. Pharmacol. Exp. Ther.,* **264,** 1352–1363.

LEFF, P. (1995). The two-state model of receptor activation. *Trends Pharmacol. Sci.,* **16,** 89–97.

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.

MUNRO, S., THOMAS, K. L. & ABU-SHAAR, M. (1993). Molecular characterization of a peripheral receptor for cannabinoids. *Nature,* **365,** 61–64.

PACHECO, M.A., WARD, S.J. & CHILDERS, S.R. (1994). Differential requirements of sodium for coupling of cannabinoid receptors to adenylyl cyclase in rat brain membranes. *J. Neurochem.,* **62,** 1773–1782.

PERT, C.B., PASTERNAK, G. & SNYDER, S.H. (1973). Opiate agonists and antagonists discriminated by receptor binding in brain. *Science,* **182,** 1359–1361.

PERTWEE, R.G., FERNANDO, S.R., GRIFFIN, G., RYAN, W., RAZDAN, R.K., COMPTON, D.R. & MARTIN B.R. (1996). Agonist-antagonist characterization of 6-cyano-2′-yne-Δ^8-tetrahydrocannabinol in two isolated tissue preparations. *Eur. J. Pharm.,* **315,** 195–201.

RINALDI-CARMONA, M., BARTH, F., HEAULME, M., SHIRE, D., CALANDRA, B., CONGY, C., MARTINEZ, S., MARUANI, J., NELIAT, G., CAPUT, D., FERRARA, P., SOUBRIE, P., BRELIERE, J.C. & LE FUR, G. (1994). SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Lett.,* **350,** 240–244.

RINALDI-CARMONA, M., BARTH, F., MILLAN, J., DEROCQ, J.M., CASELLAS, P., CONGY, C., OUSTRIC, D., SARRAN, M., BOUABOULA, M., CALANDRA, B., PORTIER, M., SHIRE, D., BRELIERE, J.C. & LE FUR, G.L. (1998). SR 144528, the first potent and selective antagonist of the CB_2 cannabinoid receptor. *J. Pharmacol. Exp. Ther.,* **284,** 644–650.

SHOWALTER, V.M., COMPTON, D.R., MARTIN, B.R. & ABOOD, M.E. (1996). Evaluation of binding in a transfected cell line expressing a peripheral cannabinoid receptor (CB_2): Identification of cannabinoid receptor subtype selective ligands. *J. Pharmacol. Exp. Ther.,* **278,** 989–999.

THOMAS, B.F., GILLIAM, A.F., BURCH, D.F., ROCHE, M.J. & SELTZMAN, H.H. (1998). Comparative receptor binding analyses of cannabinoid agonists and antagonists. *J. Pharmacol. Exp. Ther.,* **285,** 285–292.

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