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Large Receptor Reserve for Cannabinoid Actions in the Central Nervous System[1]

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ABSTRACT

The receptor occupancy required to produce cannabinoid effects in the central nervous system was determined in both a neurochemical and a behavioral assay for cannabinoid actions. In the neurochemical experiments, performed on superfused rat hippocampal slices, electrically evoked [^{3}H]acetylcholine release was inhibited by the cannabinoid agonist, WIN 55212 to 2 with an EC_{50} of 0.005 μM and maximum effect of 79%. In parallel experiments examining binding of the radiolabeled CB1 antagonist [^{131}I]AM 281 {*N*-(morpholin-4-yl)-5-(4-[^{131}I]iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide} to living hippocampal slices, WIN 55212 to 2 inhibited [^{131}I]AM 281 binding with an EC_{50} of 1.3 μM. From these two sets of data it was determined that 50% of maximal inhibition of [^{3}H]acetylcholine release in hippocampal slices occurs at a receptor occupancy of only 0.13% and 95% of maximal inhibition at a receptor occupancy of 7.5%, suggesting the presence of a receptor reserve that is large compared with other G protein-coupled receptor systems in the central nervous system. In behavioral experiments, WIN 55212 to 2 inhibited spontaneous locomotor activity in mice with an ED_{50} of 0.3 mg/kg, i.v.. In in vivo binding experiments using [^{131}I]AM 281, WIN 55212 to 2 failed to produce significant inhibition of radiotracer binding in the mouse brains, except at very high doses (10 mg/kg or greater, i.v.). By contrast, the CB1 antagonist SR 141716A (10 mg/kg, i.p.), completely abolished specific [^{131}I]AM 281 binding. These experiments suggest that behavioral effects of cannabinoids, like neurochemical effects, are produced at very low receptor occupancy.

The actions of cannabinoids, such as Δ^9-tetrahydrocannabinol, in the brain appear to be mediated predominately through a single type of receptor, termed the CB1 receptor (Herkenham et al., 1990; Matsuda et al., 1990). A second type of cannabinoid receptor, termed the CB2 receptor, is found mainly outside of the central nervous system (Munro et al., 1993). At a behavioral level, activation of brain cannabinoid receptors produces effects in animals such as analgesia, inhibition of locomotor activity, catalepsy, and hypothermia (Compton et al., 1992). At a neurochemical level, a principle function of cannabinoid receptors in the brain appears to be the presynaptic modulation of neurotransmitter release from nerve terminals. Thus, cannabinoid receptor activation has been shown to inhibit both glutamate and acetylcholine (ACh) release in the hippocampus and γ-aminobutyric acid release in the substantia nigra (Miller and Walker, 1995; Gifford and Ashby, 1996; Shen et al., 1996; Gifford et al., 1997a).

For many neurotransmitter systems in the brain it is necessary for only a fraction of the available receptors to be occupied by an agonist to produce a full functional response. A knowledge of the size of such a receptor reserve in a system is useful to be able to predict the effects of weak partial agonists. Thus in a system in which there is a large receptor reserve even agonists with very low efficacy may behave as although they are full agonists. Conversely, in a system with no receptor reserve such low efficacy compounds may be indistinguishable from antagonists. Whether a receptor reserve exists can be determined from a knowledge of the degree of receptor occupancy required by an agonist to a produce a given level of functional effect. Because in the case of the cannabinoid actions in the central nervous system this is currently unknown, we undertook the present investigation with the objective of determining this relationship. For measuring the receptor occupancies in a neurochemical system we examined the inhibition of electrically evoked [^{3}H]ACh release from hippocampal brain slices by the cannabinoid agonist, WIN 55212-2 (Gifford and Ashby, 1996; Gifford et al., 1997a). To measure the receptor occupancies in a behavioral system we examined the effects of WIN 55212-2 in inhibiting spontaneous locomotor activity in mice. WIN 55212-2 was chosen as the agonist for these experiments

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ABBREVIATIONS: ACh, acetylcholine; BSA, bovine serum albumin.

because it has a relatively high efficacy compared with other cannabinoid agonists (Sim et al., 1996; Burkey et al., 1997), and thus has the greatest likelihood of revealing whether a receptor reserve is present.

One popular method to determine receptor occupancies and receptor reserve in biological systems has been to progressively inactivate an increasing percentage of the receptors using an irreversibly binding antagonist and to measure the resultant effects on the functional dose-response curve for the ligand investigated, along with concomitant measurements of the reduction in B_{max} using an in vitro receptor binding assay. Increasing inactivation of the receptors produces a progressive rightward shift in the dose-response curve, which is subsequently followed by a depression in the maximal response. The apparent dissociation constant of the agonist can be determined from this data using the method of Furchgott and Bursztyn (1967) and receptor occupancies and reserve determined. To examine the effects of cannabinoids on neurotransmitter release and behavior in the present study we employed an alternative approach to determine receptor occupancy in which, after constructing a dose-response curve for the functional effects of the agonist, parallel experiments were conducted to measure the agonist-induced inhibition of binding of a cannabinoid radiotracer bound to the receptors in situ in either living brain slices or in the intact brain in vivo. From the dose-response curve for the functional effect and the displacement curve for inhibition of radiotracer binding a curve of receptor occupancy versus functional effect could be constructed and the receptor reserve thus determined. This approach requires the availability of a radiotracer with a sufficiently high affinity and low lipophilicity to be able to label receptors in vivo. For a radiotracer we used [^{131}I]AM 281 {*N*-(morpholin-4-yl)-5-(4-[^{131}I]iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide}, which is a less lipophilic analog of the cannabinoid antagonist, SR 141716A (Gatley et al., 1998). We previously found [^{131}I]AM 281 to be effective in labeling cannabinoid receptors in vivo in both experiments in mice and in baboon single photon emission computed tomography experiments (Gatley et al., 1998). Like SR 141716A, AM 281 shows an antagonist profile in in vitro experiments (Gifford et al., 1997b).

Materials and Methods

[^{3}H]ACh Release in Slices. Male Sprague-Dawley rats (200–350 g; Taconic, Germantown NY) were sacrificed by decapitation, their brains removed, and the hippocampus dissected out. Following dissection, 300-μm tissue slices were cut with a vibratome and the slices transferred to 2 ml of Krebs' buffer (119.5 mM NaCl, 3.3 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.2 mM, KH_2PO_4, 11 mM glucose, and 0.03 mM EDTA, pH 7.4), saturated with 95% 0_2/5% CO_2, and containing 10 μCi [^{3}H]choline. Following incubation in the [^{3}H]choline for 15 min at 37°C, the slices were transferred to 10 superfusion chambers (two slices per chamber). The slices were sandwiched between wire mesh screens positioned midway between two platinum electrodes. Slices were superfused at 37°C, at a rate of 1.6 ml/min, with oxygenated Krebs' buffer containing 1 μM physostigmine to prevent hydrolysis of the released ACh and 0.3 μM quinuclidinyl benzilate to prevent autoinhibition of release via presynaptically located muscarinic receptors. Neither of these compounds showed any direct binding to cannabinoid receptors at these concentrations (as determined using [^{131}I]AM 281 binding to intact hippocampal slices; data not shown). To evoke neurotransmitter release, the tissue slices were given three periods of electrical stimulation (Sl, S2, and S3), each of 3 min duration, beginning 150, 200, and 250 min after superfusion was started. Each stimulation period consisted of a train of unipolar pulses (60 mA, 2 ms) at a rate of 1 Hz.

Presumably because of its lipophilic nature, WIN 55212-2, appears to readily stick to the Tygon peristaltic pump tubing and the Plexiglass used to construct the superfusion chambers and this can substantially reduce the concentration of the drug reaching the slices (our unpublished observations obtained using [^{3}H]WIN 55212–2). To avoid this problem we switched the peristaltic pump tubing from the inflow side to the outflow side of the superfusion chambers and lined the chambers with Teflon tubing, which has low drug binding properties. Additionally, after being drawn into the tubing running to the superfusion chambers, the Krebs' saline-containing drug was mixed with bovine serum albumin (BSA), added via a second pump, to act as a carrier for the drug (final BSA concentration 0.13%).

WIN 55212-2 was dissolved at a concentration of 1 mg/ml in 40% β-cyclodextrin before being added to the superfusion medium. The maximal final concentrations of the cyclodextrin in the superfusion medium was 0.006%, which we previously found does not affect neurotransmitter release (Gifford and Ashby, 1996). Control (no drug) chambers were given cyclodextrin vehicle only.

Stimulation-evoked release (S1, S2, and S3) was calculated by subtracting the mean level of counts in two 4-min fractions collected immediately before stimulation from that in a 4-min fraction collected immediately after initiating stimulation. WIN 55212-2 was added to the Krebs' saline after completion of S1. To determine the effect of WIN 55212-2 on stimulation-evoked release, data was expressed as the ratio of evoked release of radioactivity before adding drug (S1) relative to the amount of evoked release after adding drug (S2 and S3). Stimulation-evoked release for S1 (i.e., before drugs) was typically 200 to 400 cpm. To avoid inaccurate S2/S1 or S3/S1 ratios from those slices showing relatively little stimulation-evoked overflow, slices having a stimulation-evoked release before drug addition of <50 cpm were excluded from the analysis. Slices showing an unstable level of basal release (>30% variation between consecutive fractions) were also excluded from the analysis.

[^{131}I]AM 281 Binding in Slices. Approximately 1 h after being cut using a vibratome, hippocampal slices were transferred to beakers containing 50 ml Krebs' saline with 0.25% BSA, 2.5 μCi [^{131}I]AM 281, and different concentrations of either WIN 55212-2, AM 251, or SR 141716A. Slices were then incubated with moderate shaking at 37°C and under a 95% O_2-5% CO_2 atmosphere. After 2 h of incubation in the [^{131}I]AM 281, slices were individually removed from the Krebs' saline and immediately homogenized with a Tissue Tearor in 5 ml ice-cold 50 mM Tris buffer (containing 0.1% BSA and 0.03 mM EDTA, pH 7.4, at 25°C), filtered through Swinex filter holders containing GF/B Whatman filters and washed further with a 10-ml ice-cold Tris buffer. The filters were then removed from the filter holders and counted for ^{131}I in a gamma counter.

Locomotor Activity in Mice. Locomotor experiments were performed on male Swiss-Webster mice (25–30 g). Mice were maintained on a 12 h light/dark cycle with lights on at 2 AM and off at 2 PM. Locomotor experiments were performed between 300 PM and 500 PM, during the mice's dark cycle, so that they would maintain relatively high levels of spontaneous activity. For the activity experiments, mice were injected via a tail vein with 0.1 ml WIN 55212-2 in 40% cyclodextrin and immediately placed in the activity monitors (San Diego Instruments, San Diego, CA) with two mice per activity monitor. Activity was measured as the total number of photocell beam interruptions over a period between 5 and 25 min after placing the mice in the monitors

[^{131}I]AM 281 Binding in Mice. Mice were injected via a tail vein with 0.2 ml of a 40% cyclodextrin solution containing [^{131}I]AM 281 (0.5 μCi/mouse) plus WIN 55212-2 (1–30 mg/kg). Animals were sacrificed by decapitation 1 h later, their brains removed, and the hippocampus, cerebellum, and brain stem dissected out. Tissue samples were weighed and assayed for ^{131}I by gamma counting.

Data Analysis. Best-fit curves through the data from the superfusion experiments, [^{131}I]AM 281 binding experiments, and locomotor activity experiments were determined using the nonlinear regression program contained in Inplot (Graphpad software, San Diego, CA). In vivo binding data in mice was analyzed using a single-factor analysis of variance followed by a Dunnett's test for comparing multiple treatment group means to a single control group mean.

Drugs. R(+)WIN 55212-2 mesylate, (±)-quinuclidinyl benzilate, and 2-hydroxypropyl-β-cyclodextrin were obtained from Research Biochemicals Inc. (Natick, MA). A23187, physostigmine (eserine), and BSA albumin were obtained from Sigma Chemical Co. (St. Louis, MO). SR 141716A was obtained from Sanofi Recherche. [^{131}I]AM 281 was prepared by radioiododestannylation of its tributyltin precursor as previously described (Lan et al., 1996; Gatley et al., 1998)

Results

In the hippocampal slices, WIN 55212-2 produced a dose-dependent inhibition of electrically evoked [^{3}H]ACh release in both S2 and S3 (Fig. 1). Electrically evoked [^{3}H]ACh release was reduced by a maximum of 74% in S2 and 79% in S3, with EC_{50}s of 0.012 μM in S2 and 0.005 μM in S3. The slightly lower EC_{50} for S3 compared with S2 was probably a consequence of the longer drug exposure time allowing a more complete equilibration of the slices with the superfusate concentration of the drug.

In the radiotracer binding studies using hippocampal slices both WIN 55212-2 produced a dose-dependent inhibition of [^{131}I]AM 281 binding (Fig. 2). The highest concentration of WIN 55212-2 reduced [^{131}I]AM 281 binding to below that of the nonspecific binding, as defined by 1 μM SR 141716A. Although this could be taken to indicate the presence of a small population of receptors in the slice that bind [^{131}I]AM 281 and WIN 55212-2 but not SR 141716A, this is probably unlikely because of the chemical similarity of AM 281 and SR 141716A. A more likely explanation is that the 1-μM concentration of SR 141716A was not quite enough to displace all of the [^{131}I]AM 281 binding. The EC_{50} and Hill slope for the displacement of [^{131}I]AM 281 binding by WIN 55212-2 (taking the [^{131}I]AM 281 binding at 10 μM WIN as representative of the maximum displacement) were 1.3 μM and 0.55, respectively. Because a tracer level of [^{131}I]AM 281 was employed for these experiments, the EC_{50} for displacement of radioligand binding would be approximately equal to the K_i for WIN 55212-2 binding to the receptors in the living slices. An accurate measurement of K_i is also facilitated by the readily reversible kinetics of AM 281(Gatley et al., 1998).

In a seperate series of experiments, we determined the potency of the cannabinoid antagonist, AM 251, in inhibiting [^{131}I]AM 281 binding in the hippocampal slices. AM 251 is chemically similar to SR 141716A except that it possesses a 4-iodophenyl group in place of the 4-chlorophenyl in SR 141716A, and it has a similar potency to SR 141716A in tissue homogenate binding assays (Gatley et al., 1998). In the hippocampal slices AM 251 was found to potently inhibit [^{131}I]AM 281 binding with an EC_{50} of 2 nM and Hill slope of 0.66 (Fig. 3).

Knowing the K_i and Hill slope for WIN 55212-2 binding in the living slices, the percent occupancy of cannabinoid receptors in the slices for any given concentration of WIN 55212-2 can be determined. If it is assumed that the receptor subtype that binds the [^{131}I]AM 281 (i.e., the CB1 receptor) also mediates the action of WIN 55212-2 on hippocampal ACh release, then the receptor occupancy to produce a given degree of inhibition of [^{3}H]ACh release can be calculated (Fig. 4). From this figure it can be determined that the half-

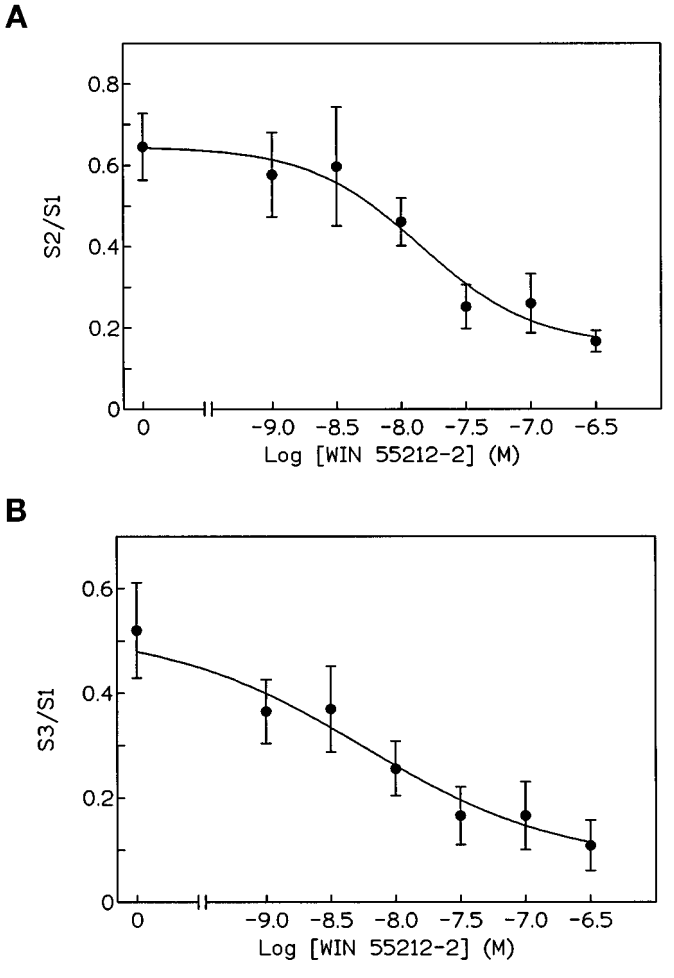

Fig. 1. Effect of WIN 55212-2 on the electrically evoked release of [^{3}H]ACh from hippocampal slices after either 40 min exposure to WIN 55212-2 (S2/S1 ratio) (A) or 90 min exposure to WIN 55212-2 (S3/S1 ratio) (B). Data are means (±S.E.M.) of 9 to 22 observations.

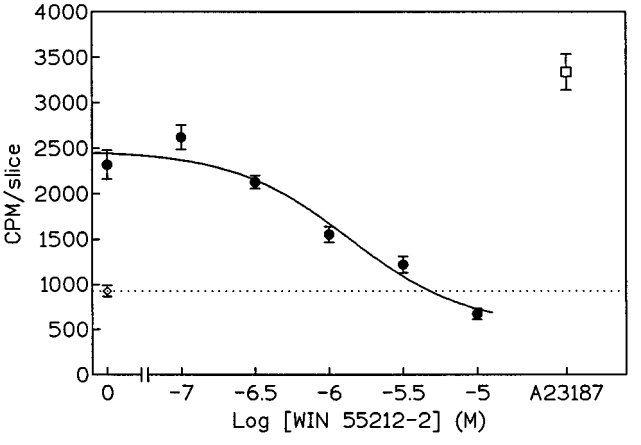

Fig. 2. Inhibition of [^{131}I]AM 281 binding in hippocampal slices by WIN 55212-2 (●). SR 141716A (1 μM) (··· ◇ ···) was used to determine the level of nonspecific binding. Also shown is the effect of 2 μM A23187 (□). Data are means (±S.E.M.) of 5 to 20 observations.

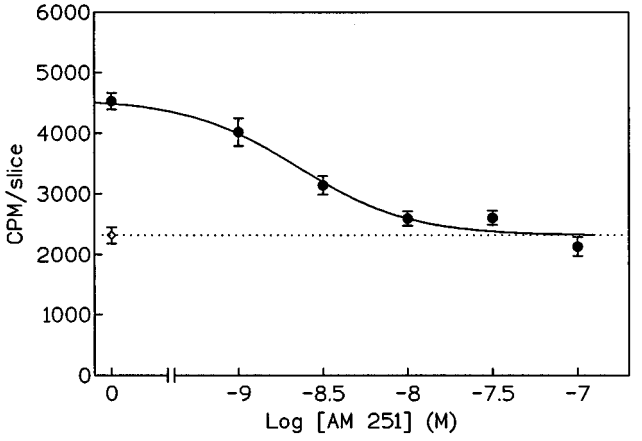

Fig. 3. Inhibition of [^{131}I]AM 281 binding in hippocampal slices by AM 251 (●). AM 251 (1 μM) (··· ◇ ···) was used to determine the level of nonspecific binding. Data are means (±S.E.M.) of 6 to 13 observations.

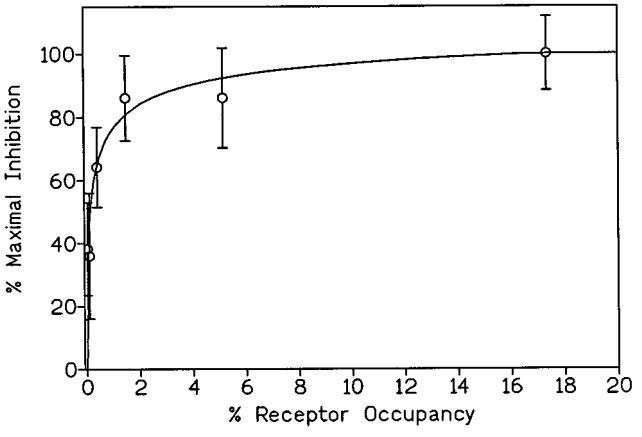

Fig. 4. Inhibition of [^{3}H]ACh release by WIN 55212-2 plotted as a function of receptor occupancy using data from Figs. 1B and 2. The curve was calculated from the equation parameters (EC_{50} and Hill slope) for the nonlinear regression curves drawn through the data in these figures.

maximal inhibition of [^{3}H]ACh release occurs at a receptor occupancy of 0.13%, and a 95% of maximal inhibition of [^{3}H]ACh release occurs at a receptor occupancy of 7.5%.

In addition to examining the effect of WIN 55212-2 on [^{131}I]AM 281 binding, the effect of the calcium ionophore A23187 in the slices was also examined to determine whether the slices were able to release endogenous ligands capable of inhibiting [^{131}I]AM 281 binding. This ionophore has been found to cause the release of a variety of neurotransmitters from neural tissue, including the putative endogenous cannabinoid receptor ligand, anandamide (Di Marzo et al., 1994). A23187 (2 μM) was added 30 min before homogenizing and filtering the slices. In the presence of A23187 the radiotracer binding, rather than being decreased by the release of endogenous ligands, appeared to be slightly increased (Fig. 2).

To examine the effects of WIN 55212-2 in a behavioral assay, we examined the action of this compound on spontaneous locomotor activity in mice. When administered to the mice, WIN 55212-2 produced catalepsy within approximately 1 min after the injection. Spontaneous locomotor activity, measured as the total number of photocell beam breaks over a 5- to 25-min period after the time of injection, was concomitantly reduced (Fig. 5). The ED_{50} for the effects of WIN 55212-2 in this assay was 0.3 mg/kg, i.v..

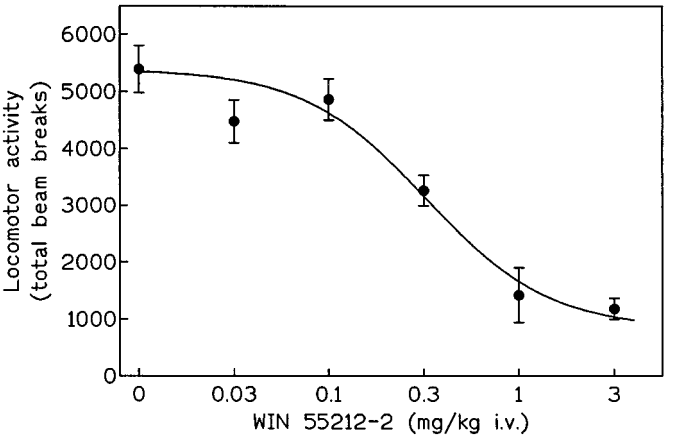

Fig. 5. Inhibition of spontaneous locomotor activity in mice by WIN 55212-2. Data are means (±S.E.M.) of 6 to 10 mice.

The effect of different i.v. doses of WIN 55212-2 on the in vivo binding of [^{131}I]AM 281 in the mice was also examined. Binding of the radiotracer was measured in both the hippocampus and cerebellum, which possess high densities of cannabinoid receptors (Herkenham et al., 1990), whereas the brain stem, which possesses relatively few cannabinoid receptors, was used as a control region (Fig. 6). In both the hippocampus and cerebellum, specific [^{131}I]AM 281 binding was relatively unaffected by WIN 55212-2, except at very high doses (10 and 30 mg/kg, i.v.), where binding was significantly reduced. By contrast, SR 141716A, at a dose of 10 mg/kg, i.p., completely inhibited specific [^{131}I]AM 281 binding in these brain regions.

Discussion

WIN 55212-2 produced a dose-dependent inhibition of the electrically evoked [^{3}H]ACh release from hippocampal slices. The maximum inhibition of electrically evoked hippocampal [^{3}H]ACh release observed in the present study (79%) was similar to the degree of inhibition of hippocampal ACh release by cannabinoid agonists we have seen in previous studies (Gifford and Ashby, 1996; Gifford et al., 1997a). However, the EC_{50} for WIN 55212-2 in the present study was slightly lower than that obtained in a previous study (Gifford and Ashby, 1996), most likely because of the inclusion of BSA to act as a carrier for the drug, as well as changes made to the apparatus to reduce drug binding to the walls of the tubing and to the superfusion chambers (see *Materials and Methods*).

In the binding experiments on hippocampal slices, WIN 55212-2 displaced the tracer level of [^{131}I]AM281 binding with a K_i of 1.3 μM. Because the WIN 55212-2 is binding to receptors on living cells in this preparation, the K_i obtained in the present study will not necessarily be the same as that obtained for radiotracer displacement in a homogenate binding study, because the relative proportions of high and low affinity states of the receptor may be different in the two cases. This is because of the different environments of the receptor and different levels of GTP (probably higher in living cells than in washed homogenate preparations). Previous homogenate binding studies have given K_i values for WIN

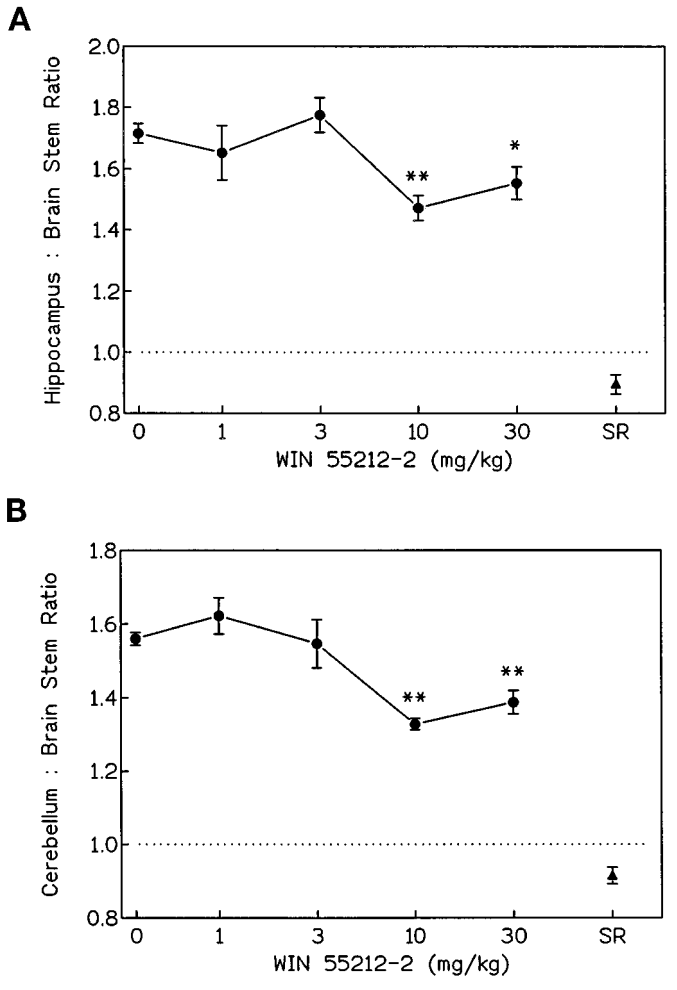

Fig. 6. Effect of WIN 55212-2 and SR 141716A on the in vivo binding of [^{131}I]AM 281 in mice in hippocampus (A) or cerebellum (B). Data are means (±S.E.M.) of 5 to 10 mice. *$p < .05$, **$p < .01$ using Dunnett's test. ANOVA (excluding SR 141716A group): $F(4,28) = 5.6$, $p < .05$ for hippocampus and $F(4,28) = 10.9$, $p < .01$ for cerebellum.

55212-2 displacement of radiolabeled antagonist ([^{3}H]SR 141716A or [^{123}I]AM 281) binding of 0.03 to 0.08 μM (Petitet et al., 1997; Gatley et al., 1998), suggesting a higher proportion of high affinity binding sites in homogenate preparations than for the receptors in situ. In the case of AM 251, its potency in the rat brain slices was quite similar to that we have previously determined for this compound in homogenate binding studies performed using mice brain tissue (K_i, 6 nM; Gatley et al., 1998). This is to be expected for this compound because since it is an antagonist it will not be affected by differences in high and low agonist affinity states of the receptor.

From the [^{131}I]AM 281 binding experiments the percent occupancy of cannabinoid receptors for a given concentration of WIN 55212-2 could be determined. Assuming that the same receptor type binds the [^{131}I]AM 281 as inhibits the release of ACh (which is probable because only CB1 mRNA has been identified with certainty in the brain), then the receptor occupancy to produce a given degree of inhibition of [^{3}H]ACh release can be determined. This analysis suggested that the EC_{50} for inhibition of [^{3}H]ACh release by WIN 55212-2 was reached with a receptor occupancy of only 0.13%, whereas 95% of the maximal effect was reached with a receptor occupancy of 7.5%, indicating the presence of a substantial receptor reserve. The receptor reserve for presynaptically located cannabinoid receptors in this system appears to be larger than that determined for most other G protein-coupled receptor systems in the central nervous system. For example, in the case of the dopamine receptor-mediated reversal of γ-butyrolactone-induced striatal L-dopa accumulation (Meller et al., 1986), the inhibition of firing of A9 dopamine neurons in the substantia nigra by dopamine autoreceptors (Cox and Waszczak, 1990), or the inhibition of locus ceruleus neurons by *alpha* adrenergic receptors (Pineda et al., 1997), receptor reserves of 70 to 90% have been documented with, in the latter two cases, 50% of maximal functional effect occurring at approximately 4% receptor occupancy (Cox and Waszczak, 1990; Pineda et al., 1997). In other G protein-coupled receptor systems, for example the inhibition of ACh release in the hippocampus by presynaptic muscarinic autoreceptors (Vickroy et al., 1993) or the dopamine receptor-mediated elevation of in vivo striatal ACh levels (Enz et al., 1990), no receptor reserve is apparent.

A large receptor reserve for cannabinoid receptors on presynaptic terminals is consistent with the ability of SR 141716A and AM 281 to potentiate electrically evoked ACh release when added on their own to hippocampal slices (Gifford and Ashby, 1996; Gifford et al., 1997b). Thus, if cannabinoid receptors possessed a small degree of activity without agonist binding (constitutive activity), a large receptor reserve would mean that even low levels of such activity may be sufficient to appreciably activate second messenger systems and thus produce a tonic level of suppression of ACh release. Under these conditions, SR 141716A and AM 281 would cause an enhancement of ACh release if they are acting as inverse agonists, as has been suggested by recent data (Landsman et al., 1997).

In a recent report by Breivogel et al. (1997), the [^{35}S]GTPγS binding assay in tissue sections was used to calculate receptor/transducer amplification factors for cannabinoid receptors. For the hippocampus, a value of approximately 2 was obtained for the ratio of the number of G proteins activated per agonist-occupied cannabinoid receptor in hippocampal tissue. However, how this translates into producing a given level of physiological responses in the tissue for a particular degree of receptor occupancy will depend additionally on downstream factors such as the affinity and relative concentrations in the membrane of cannabinoid activated G proteins relative to adenylate cyclase enzyme.

In the behavioral experiments, WIN 55212-2 inhibited spontaneous locomotor activity in mice with an ED_{50} of 0.3 mg/kg i.v. This compares with an ED_{50} value of 0.13 mg/kg i.v. obtained by (Compton et al., 1992) for the effect of WIN 55212-2 on spontaneous locomotor activity in mice. In two other behavioral assays for cannabinoid action, the drug discriminative stimulus and antinociception in the tail-flick assay, ED_{50} values for WIN 55212-2 are in approximately the same range as those obtained in locomotor assays (Compton et al., 1992).

The in vivo binding experiments indicated that doses of WIN 55212-2, which produced a strong suppression of locomotion in the behavioral assay, had a negligible effect on hippocampal and cerebellar [^{131}I]AM 281 binding. In fact, even when given at very much higher doses, which were possible because of the low toxicity of cannabinoid agonists,

WIN 55212-2 appeared relatively ineffective in reducing specific [^{131}I]AM 281 binding to these brain areas. This was in contrast to SR 141716A, which effectively prevented specific [^{131}I]AM 281 binding to these brain areas. The relative ineffectiveness of WIN 55212-2 in inhibiting [^{131}I]AM 281 binding compared with SR 141716A may indicate that, as suggested in the hippocampal slices, most of the cannabinoid receptors in vivo are in a low agonist affinity state. These results also suggest that, similar to the functional effects of cannabinoids in the hippocampal slices, the receptor occupancy by WIN 55212-2 needed to evoke behavioral effects is very low.

In conclusion, the results of the present study suggest that a substantial receptor reserve exists for both the neurochemical and behavioral effects of cannabinoids. One consequence of a large receptor reserve is that even low efficacy partial agonists will be able to produce a full functional effect in the animal. Thus, anandamide has a relatively low intrinsic efficacy compared with WIN 55212-2 in its ability to activate G proteins (Burkey et al., 1997a). However, in the hippocampal slices, we found that anandamide, in the presence of an amidase inhibitor (palmitylsulfonyl fluoride; AM 374) to prevent breakdown, can produce a maximal inhibition of ACh release that is similar to that obtained with WIN 55212-2 (manuscript in preparation). Similarly, Δ^{9}-tetrahydrocannabinol, which has also been found to have a relatively low intrinsic efficacy in terms of its ability to activate G proteins (Sim et al., 1996; Burkey et al., 1997b), produces similar maximal effects as WIN 55212-2 in behavioral tests for antinociception, catalepsy, motor activity, and drug discrimination (Compton et al., 1992).

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