Regional Differences in Cannabinoid Receptor/G-protein Coupling in Rat Brain

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ABSTRACT

Cannabinoid receptor activation of G-proteins can be measured by WIN 55212–2-stimulated [35S]GTPγS binding. Receptor/transducer amplification factors, interpreted as the number of G-proteins activated per occupied receptor, are the ratio of the apparent Bmax of net agonist-stimulated [35S]GTPγS binding to the Bmax of receptor binding. The present study examined whether amplification factors for cannabinoid receptors differ among various rat brain regions. In autoradiographic studies with [3H]WIN 55212–2 and WIN 55212–2-stimulated [35S]GTPγS binding, some regions displayed different relative levels of agonist-stimulated [35S]GTPγS binding than receptor binding. To quantify amplification factors, membranes from different brain regions were assayed by saturation binding analysis of net WIN 55212–2-stimulated [35S]GTPγS, [3H]SR141716A (antagonist) and [3H]WIN 55212–2 (agonist) binding. For [3H]SR141716A binding, amplification factors varied significantly from 2.0 (frontal cortex) to 7.5 (hypothalamus). For [3H]WIN 55212–2 binding, amplification factors ranged from 2.4 (hippocampus) to 5.5 (thalamus). Comparison of receptor binding and G-protein activation at subsaturating concentrations of WIN 55212–2 indicated that amplification factors may vary with receptor occupancy in some regions like cerebellum. Ratios between high-affinity [3H]WIN 55212–2 Bmax and [3H]SR141716A Bmax also differed significantly among brain regions. These results demonstrate that G-protein coupling by cannabinoid receptors differs among brain regions, and therefore depends on the cellular environment.

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ABBREVIATIONS: CB1, brain cannabinoid receptor subtype; GTPγS, guanosine 5’-O-(3-thiotriphosphate); BSA, bovine serum albumin; EGTA, ethylenedinitrilo]tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid; ANOVA, analysis of variance.
of cannabinoid receptor binding sites than either type of opioid receptor. Therefore, each cannabinoid receptor activated only one seventh as many G-proteins as each mu or delta opioid receptor (Sim et al., 1996c).

The choice of radioligand used for receptor binding assays is critical. In our previous studies, amplification factors were calculated using high-affinity agonist ([3H]WIN 55212–2) binding (Sim et al., 1996c). However, such binding cannot be completely correlated with agonist-activated G-proteins because high-affinity agonist binding cannot be measured under the same assay conditions (with sodium and GDP) (Devane et al., 1988) as agonist-stimulated [35S]GTPγS binding. One of the important goals of the present study was to compare values of amplification factors calculated by high-affinity agonist receptor binding with those calculated from total receptor binding (determined with 3H-labeled antagonist).

The recent synthesis of SR141716A as a selective CB1 antagonist (Rinaldi-Carmona et al., 1994), and the development of [3H]SR141716A as an antagonist radioligand for CB1 receptors (Rinaldi-Carmona et al., 1996), allowed for this opportunity, because [3H]SR141716A binding is unaffected by sodium, magnesium or guanine nucleotides (Rinaldi-Carmona et al., 1996).

Previous autoradiographic analysis of rat brain sections has revealed that cannabinoid receptors (determined by [3H]WIN 55212–2 binding) and cannabinoid activation of G-proteins (determined by net WIN 55212–2–stimulated [35S]GTPγS) have similar distributions (Sim et al., 1995; Childers and Deadwyler, 1996). However, some differences were observed: some regions with relatively low receptor density exhibited high levels of G-protein activation, whereas other regions with similar receptor densities exhibited low levels of G-protein activation. To further investigate this observation quantitatively, the present study compared saturation binding analyses of cannabinoid receptor-stimulated [35S]GTPγS binding and receptor binding in membrane homogenates from 10 different rat brain regions. [35S]GTPγS binding was determined in the presence of maximally effective concentrations of WIN 55212–2, and receptor density and affinity were measured with both the agonist, [3H]WIN 55212–2, and the antagonist, [3H]SR141716A. These results confirm that the efficiency of cannabinoid receptor coupling to G-proteins differs throughout the brain. In this study, the receptor/transducer amplification factor is defined as the apparent Bmax of net agonist-stimulated [35S]GTPγS binding divided by the Bmax of cannabinoid receptor ligand binding sites. The fraction of cannabinoid agonist high-affinity binding sites is defined as the Bmax of 3H-labeled agonist ([3H]WIN 55212–2) binding divided by the Bmax of 3H-labeled antagonist ([3H]SR141716A) binding.

Materials and Methods

Materials. Male Sprague-Dawley rats were purchased from Zivic Miller (Zeleinople, PA). [35S]GTPγS (1000–1250 Ci/mmol), [3H]WIN 55212–2 (45.5 Ci/mmol) and Reflections™ film were purchased from New England Nuclear Corp. (Boston, MA). [3H]SR141716A (43–65 Ci/mmol) and Hyperfilm βmax were obtained from Amersham Life Sciences (Arlington Heights, IL). WIN 55212–2 was purchased from Research Biochemicals International (Natick, MA). SR141716A was a generous gift from Dr. Francis Barth at Sanofi Recherche (Montpellier, France). GDP for membrane [35S]GTPγS binding assays and GTPγS were purchased from Boehringer Mannheim (New York, NY). GDP for [35S]GTPγS autoradiography was purchased from Sigma Chemical Co. (St. Louis, MO). Ecolite scintillation fluid was obtained from ICN (Irvine, CA). All other reagent grade chemicals were obtained from Sigma Chemical Co. or Fisher Scientific (Pittsburgh, PA).

[35S]GTPγS autoradiography. Animals were sacrificed by decapitation, and the brains were removed and immersed in isopentane at –35°C. Twenty-micron coronal sections were cut on a cryostat and thaw-mounted onto gelatin-coated slides. Slides were rinsed in assay buffer (50 mM Tris-HCl, 3 mM MgCl2, 0.2 mM EGTA, 100 mM NaCl, 0.1% (w/v) BSA, pH 7.4) at 25°C for 10 min. Slides were then incubated with 2 mM GDP in assay buffer at 25°C for 15 min. Sections were incubated with [35S]GTPγS (0.04 nM) and 2 mM GDP, with 10 μM WIN 55212–2 in assay buffer at 25°C for 2 hr. Basal [35S]GTPγS binding was assessed in the absence of agonist. Slides were rinsed twice in cold 50 mM Tris buffer and once in deionized water, dried and exposed to film for 48 hr. Films were digitized with a Sony XC-77 video camera and analyzed with the NIH Image program for Macintosh computers. Images were quantified by densitometric analysis with [35S] standards. Values are expressed as femtomoles of radioligand bound/mg tissue and corrected for [%] based on incorporation of [%] into brain paste standards (Sim et al., 1996b).

[3H]WIN 55212–2 receptor autoradiography. Brains were processed as described above and stored at –80°C until use. Slides were brought to room temperature and preincubated in assay buffer (20 mM HEPES with 0.5% (w/v) BSA and 1 mM MgCl2) for 20 min at 30°C. Slides were incubated in 1 nM [3H]WIN 55212–2 in assay buffer for 80 min at 30°C. Nonspecific binding was assessed in the presence of 1 μM WIN 55212–2. Slides were rinsed four times for 10 min each in assay buffer at 25°C, then twice in deionized water at 4°C. Slides were dried thoroughly and exposed to Hyperfilm βmax for 3 weeks. Films were analyzed as described above. [3H] standards were used for quantification and values are expressed as femtomoles of radioligand bound/mg tissue.

Membrane preparations. Ten brain regions were dissected from fresh rat brains on ice. Tissue samples were pooled and homogenized with a Tissumizer (Tekmar, Cincinnati, OH) in cold membrane buffer (50 mM Tris-HCl, pH 7.4, 3 mM MgCl2, 1 mM EGTA) and centrifuged at 31,000 × g for 10 min at 4°C. Pellets were resuspended in membrane buffer, then centrifuged at 31,000 × g for 10 min at 4°C. Pellets were homogenized in membrane buffer, assayed for protein content (Bradford 1976) and stored in aliquots at –80°C until being assayed.

Agonist-stimulated [35S]GTPγS binding assays. Frozen membranes were thawed and diluted in membrane buffer, and centrifuged at 48,000 × g for 4 min. Pellets were resuspended and homogenized in cold assay buffer (50 mM Tris-HCl pH 7.4, 3 mM MgCl2, 0.2 mM EGTA, 100 mM NaCl), then assayed for protein (Bradford, 1976). For saturation binding analysis, membranes were incubated for 1 hr with 0.5 to 15 nM unlabeled GTPγS in the presence or absence of 3 μM WIN 55212–2. WIN 55212–2 ED50 and SR141716A K, values were determined by incubating membranes for 2 hr with various concentrations of WIN 55212–2 (10–30,000 nM) in the presence and absence of 2 nM SR141716A. All assays included 10 to 20 μg of membrane protein and were conducted at 30°C with 0.1% (w/v) BSA, 30 μM GDP and 0.05 nM [3H]GTPγS in a final volume of 1 ml. Nonspecific binding was determined in the absence of WIN 55212–2 and the presence of 30 μM unlabeled GTPγS. Reactions were terminated by rapid filtration under vacuum through Whatman GF/B glass fiber filters, followed by three washes with cold Tris buffer, pH 7.4. Bound radioactivity was determined by liquid scintillation spectrophotometry at 95% efficiency for [%] after overnight extraction of the filters in 4 ml Ecolite scintillation fluid.

[3H]SR141716A receptor binding assays. Membranes were prepared and incubated under the same conditions as for the [35S]GTPγS binding assays. Saturation binding analyses were performed by varying the concentration of [3H]SR141716A (0.02–2 nM)
and incubating for 1 hr, and nonspecific binding was determined with 1 μM unlabeled SR141716A. IC<sub>50</sub> values for WIN 55212–2 were determined by varying the concentration of WIN 55212–2 (10–30,000 nM) in the presence of 0.5 nM [3H]SR141716A and incubating for 2 hr. All binding assays included 3 to 10 μg of membrane protein and were conducted in [35S]GTP<sup>S</sup> binding assay buffer (as described above), including 0.1% (w/v) BSA and 30 μM GDP. Assay tubes were incubated at 30°C and binding was terminated and bound radioactivity determined (at 45% efficiency for [3H]) as above.

**[3H]WIN 55212–2 receptor binding assays.** Frozen membranes were thawed and diluted in membrane buffer, and centrifuged at 48,000 × g at 4°C for 10 min. Pellets were resuspended and homogenized in 7 ml cold 20 mM HEPES-HCl, pH 8.0, with 1 mM MgCl<sub>2</sub>, and assayed for protein (Bradford 1976). Saturation binding analyses were performed with 1 nM [3H]WIN 55212–2 plus 0.5 to 15 nM unlabeled WIN 55212–2 in a final volume of 1 ml including 0.1% (w/v) BSA. Nonspecific binding was determined in the presence of 4 μM WIN 55212–2. Assay tubes were incubated at 25°C for 90 min, and binding was terminated by rapid filtration under vacuum through Whatman GF/B glass fiber filters, followed by three washes with cold 20 mM HEPES-HCl, pH 8.0 buffer containing 1 mM MgCl<sub>2</sub> and 0.05% (w/v) BSA. Bound radioactivity was determined as above.

**Data analysis.** Net agonist-stimulated [35S]GTP<sup>S</sup> binding values were calculated by subtracting basal binding values (absence of agonist) from agonist-stimulated values at each concentration of unlabeled GTP<sup>S</sup>. Binding analysis (including receptor binding, [35S]GTP<sup>S</sup> saturation and agonist concentration-effect curves) was conducted by nonlinear regression with use of JMP for Macintosh (SAS, Cary, NC), or LIGAND (Munson and Rodbard, 1980). Mean amplification factors were calculated by dividing mean net agonist-stimulated [35S]GTP<sup>S</sup> binding apparent B<sub>max</sub> values by mean receptor binding B<sub>max</sub> values for each receptor ligand. Standard error for each amplification factor value was calculated as the square root of the variance as estimated by the equation:

\[
\text{Var}_{x} = \frac{(s_{1})^{2}}{(x_{2})^{2}} + \frac{(s_{1})^{2} \times (x_{2})^{2}}{(x_{2})^{2}}
\]

where x1 and x2 are the mean B<sub>max</sub> values for G-proteins and receptors, respectively, and s1 and s2 are the respective B<sub>max</sub> standard error values. High-affinity fraction values ([H]-labeled antagonist/[H]-labeled agonist binding B<sub>max</sub> ratios) and standard error for each region were calculated in the same manner. The Ke<sub>S</sub> value for SR141716A was calculated by the equation: K<sub>e</sub> = [Ant]/[dose-ratio − 1] (Gaddum, 1957), where [Ant] is the concentration of SR141716A and dose-ratio is the ratio of WIN 55212–2 ED<sub>50</sub> values in the presence and absence of SR141716A, respectively. Significant differences in regional amplification factor and fraction of high-affinity agonist binding values were determined conservatively by performing multiple Student’s t-tests to compare each region with every other at a significance level of α = .005 (Bonferroni adjustment to α = .05 for 10 groups). Significant differences (P < .05) between other values were determined with JMP to perform Student’s t-test for two groups, or analysis of variance and the Tukey-Kramer HSD test for multiple comparisons. Significant differences are indicated in the figures and graphs by letters: values that are not significantly different are denoted by the same single letter. Unless otherwise indicated, all data presented are the mean ± S.E.M. of three or more determinations from assays performed in triplicate.

**Results**

**Regional differences in cannabinoid receptor binding and activated G-proteins by autoradiography.** Previous autoradiographic studies have demonstrated that the distribution of cannabinoid-stimulated [35S]GTP<sup>S</sup> binding in brain correlates with that of cannabinoid receptor binding (Herkenham et al., 1991b; Sim et al., 1995). For example, both receptor- and agonist-stimulated [35S]GTP<sup>S</sup> autoradiography revealed the highest levels of receptor binding and activated G-proteins in the substantia nigra, entopeduncular nucleus and globus pallidus (Herkenham et al., 1991a; Sim et al., 1996a). To directly compare these two parameters, cannabinoid receptor ([3H]WIN 55212–2) and agonist-stimulated [35S]GTP<sup>S</sup> binding were compared in the hippocampus, amygdala, thalamus, hypothalamus and cortex in coronal rat brain sections. Representative brain sections showing specific [3H]WIN 55212–2 binding and net WIN 55212–2–stimulated [35S]GTP<sup>S</sup> binding are shown in coronal sections in figure 1. For both receptor binding and [35S]GTP<sup>S</sup> binding could be observed in other regions. Intermediate levels of receptor binding were found in the thalamus, whereas the same area provided very low levels of agonist-stimulated [35S]GTP<sup>S</sup> binding. In contrast, both amygdala and hypothalamus demonstrated intermediate-to-high levels of agonist-stimulated [35S]GTP<sup>S</sup> binding, but only low levels of cannabinoid receptor binding. A particularly striking difference between cannabinoid receptor binding and activated G-proteins was found in the cortex, which showed an intermediate level of cannabinoid receptor binding, but high levels of agonist-stimulated [35S]GTP<sup>S</sup> binding, particularly in the deeper layers of cortex. These results indicated that regional differences exist in the amplification of G-protein activity by cannabinoid receptors in the rat brain. However, it is important to note that these studies were conducted with only one concentration each of [3H]WIN 55212–2 or [35S]GTP<sup>S</sup>, and cannot provide accurate determinations of the absolute ratio between receptors and agonist-activated G-proteins.

**Regional differences in cannabinoid receptor binding and activated G-proteins in membranes.** When incubated in the presence of 30 μM GDP, WIN 55212–2 significantly increased the binding of [35S]GTP<sup>S</sup> to rat brain membranes in a concentration-dependent and saturable
manner in all regions examined: $[^{35}S]GTP\gamma S$ binding was maximal in the presence of 3 μM WIN 55212–2 and was increased by 73 to 250% in all 10 regions, similar to previous results (Selley et al., 1996). Saturation binding analysis of the binding of $[^{35}S]GTP\gamma S$ to cerebellar membranes in the presence and absence of WIN 55212–2 revealed that $[^{35}S]GTP\gamma S$ bound with high (3 nM) and low (500–1000 nM) affinity. The agonist increased the number of high-affinity sites when added in the presence of micromolar concentrations of GDP (data not shown), analogous to the agonist effect observed in the mu and delta opioid systems (Breivogel et al., 1997). To isolate the effect of the agonist on the apparent $K_D$ and $B_{max}$ of $[^{35}S]GTP\gamma S$ binding, basal binding was subtracted from agonist-stimulated binding at each concentration of GTP\gamma S. This method, which results in linear (monophasic) Scatchard plots, has been shown to yield values that correspond to the high-affinity binding of GTP\gamma S to receptor-activated G-proteins (Breivogel et al., 1997; Selley et al., 1997). To quantify differences in rat brain regional amplification factors, membrane saturation binding analyses of both cannabinoid receptors and cannabinoid receptor-activated G-proteins were conducted. Previous studies have shown that the number of G-proteins activated by a receptor can be determined by calculating the receptor/transducer amplification factor, i.e., the ratio between the $B_{max}$ value for agonist-stimulated $[^{35}S]GTP\gamma S$ binding and the $B_{max}$ for receptor binding (Sim et al., 1996c). However, determination of $B_{max}$ values for $[^{35}S]GTP\gamma S$ binding is not simple because of the necessity for GDP and a lack of clear equilibrium conditions for the assay (see “Discussion”). For this reason, parameters are termed “apparent $K_D$” and “apparent $B_{max}$” for agonist-stimulated $[^{35}S]GTP\gamma S$ binding.

Figure 2 depicts typical Scatchard plots of $[^{3}H]$SR141716A, $[^{3}H]$WIN 55212–2 and net WIN 55212–2-stimulated $[^{35}S]GTP\gamma S$ saturation binding in two representative brain regions, frontal cortex (fig. 2A) and thalamus (fig. 2B). $[^{35}S]GTP\gamma S$ apparent $B_{max}$ and $K_D$ values were similar in frontal cortex and thalamus, but both the $[^{3}H]$SR141716A and $[^{3}H]$WIN 55212–2 $B_{max}$ values were different between these two regions. For both $[^{3}H]$SR141716A and $[^{3}H]$WIN 55212–2 binding, the $B_{max}$ values were much closer to those for $[^{35}S]GTP\gamma S$ binding in the frontal cortex than in the thalamus. From these data, it is clear that the amplification factor is higher in the thalamus (3.1 for $[^{3}H]$SR141716A and 6.8 for $[^{3}H]$WIN 55212–2) than in the frontal cortex (approximately 2 for both $[^{3}H]$SR141716A and $[^{3}H]$WIN 55212–2). Another parameter, the fraction of high-affinity agonist binding, was defined as the ratio between high-affinity $[^{3}H]$WIN 55212–2 $B_{max}$ and $[^{3}H]$SR141716A $B_{max}$ values for a given region. Figure 2 also illustrates differences in the fractions of high-affinity binding between the frontal cortex and thalamus, with no significant difference between $[^{3}H]$WIN 55212–2 $B_{max}$ and $[^{3}H]$SR141716A $B_{max}$ in the frontal cortex (P = .23), but a 2-fold difference in the thalamus (P = .01).

In all 10 regions examined, both net agonist-stimulated $[^{35}S]GTP\gamma S$ and $[^{3}H]$SR141716A binding Scatchard plots were monophasic. However, in several regions, including thalamus, colliculi, sensomotor cortex, amygdala and hypothalamus (data not shown), $[^{3}H]$WIN 55212–2 also displayed some lower affinity binding ($K_D$ > 100 nM) to uncoupled CB1 receptors, resulting in biphasic Scatchard plots. All results from $[^{3}H]$WIN 55212–2 binding refer to high-affinity sites as calculated by LIGAND, but for this reason, estimates of $K_D$ and $B_{max}$ values for high-affinity $[^{3}H]$WIN 55212–2 may be somewhat less reliable than those determined for $[^{3}H]$SR141716A or net WIN 55212–2-stimulated $[^{35}S]GTP\gamma S$ binding.

Table 1 provides $K_D$ and $B_{max}$ values for CB1 receptor binding in all 10 regions. $[^{3}H]$SR141716A cannabinoid receptor binding showed no significant regional differences in $K_D$ (ANOVA, P = .15), which had a mean of 0.26 ± 0.03 nM. In $[^{35}S]GTP\gamma S$ binding assays, the $K'_D$ of unlabeled SR141716A for antagonizing WIN 55212–2-stimulated binding was $0.082 ± 0.008$ nM (data not shown), similar to the $K_D$ values of $[^{3}H]$SR141716A binding; $K_D$ values also showed no significant differences across regions. For $[^{3}H]$SR141716A binding, there were many significant differences in $B_{max}$ (ANOVA, P < .0001) values. $[^{3}H]$SR141716A $B_{max}$ values ranged from 2.5 ± 0.4 pmol/mg in the brainstem and hypothalamus to 6.9 ± 0.7 pmol/mg in the striatum. High-affinity $[^{3}H]$WIN 55212–2 binding showed many significant differences among
TABLE 1
Equilibrium cannabinoid receptor binding in membranes from various rat brain regions

<table>
<thead>
<tr>
<th>Region</th>
<th>Apparent KD</th>
<th>Apparent Bmax</th>
</tr>
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<tbody>
<tr>
<td>Frontal cortex</td>
<td>3.20 ± 0.141</td>
<td>4.58 ± 1.39</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.19 ± 0.008</td>
<td>4.67 ± 0.58</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.367 ± 0.112</td>
<td>2.76 ± 0.25</td>
</tr>
<tr>
<td>Striatum</td>
<td>0.389 ± 0.083</td>
<td>3.44 ± 0.27</td>
</tr>
<tr>
<td>Sensormotor cortex</td>
<td>0.209 ± 0.018</td>
<td>2.89 ± 0.22</td>
</tr>
<tr>
<td>Thalamus</td>
<td>0.224 ± 0.011</td>
<td>4.21 ± 0.49</td>
</tr>
<tr>
<td>Colliculi</td>
<td>0.302 ± 0.057</td>
<td>9.19 ± 0.61</td>
</tr>
<tr>
<td>Brainstem</td>
<td>0.257 ± 0.042</td>
<td>4.84 ± 0.89</td>
</tr>
<tr>
<td>Amygdala</td>
<td>0.145 ± 0.017</td>
<td>2.58 ± 0.57</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>0.134 ± 0.012</td>
<td>3.02 ± 0.15</td>
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</table>

Table 2
Saturation binding analysis of cannabinoid receptor-stimulated [35S]GTPγS binding in membranes from various rat brain regions

<table>
<thead>
<tr>
<th>Region</th>
<th>Apparent KD</th>
<th>Apparent Bmax</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal cortex</td>
<td>3.45 ± 0.46</td>
<td>10.5 ± 0.73</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>2.49 ± 0.26</td>
<td>13.1 ± 1.27</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>2.73 ± 0.19</td>
<td>12.1 ± 1.00</td>
</tr>
<tr>
<td>Striatum</td>
<td>2.26 ± 0.62</td>
<td>14.4 ± 0.75</td>
</tr>
<tr>
<td>Sensormotor cortex</td>
<td>3.00 ± 1.02</td>
<td>7.5 ± 1.49</td>
</tr>
<tr>
<td>Thalamus</td>
<td>2.09 ± 0.25</td>
<td>8.4 ± 0.83</td>
</tr>
<tr>
<td>Colliculi</td>
<td>3.10 ± 0.16</td>
<td>10.2 ± 1.52</td>
</tr>
<tr>
<td>Brainstem</td>
<td>3.08 ± 0.87</td>
<td>9.6 ± 0.90</td>
</tr>
<tr>
<td>Amygdala</td>
<td>2.69 ± 0.55</td>
<td>14.7 ± 1.89</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>2.98 ± 0.68</td>
<td>18.5 ± 0.48</td>
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regions for Bmax (ANOVA, P < 0.0001). However, only one region (colliculi) displayed a high-affinity KD value for [3H]WIN 55212-2 that was significantly different from the other regions. The high affinity Bmax values ranged from 1.2 ± 0.2 pmol/mg in the thalamus to 6.2 ± 0.6 pmol/mg in the striatum. The high-affinity KD for the colliculi was 9.2 ± 0.6 nM, and the mean high-affinity KD of the remaining regions was 3.7 ± 0.2 nM. Although Bmax values varied across regions, CB1 receptors were distributed with high density in every brain region measured with Bmax values in the picomole per milligram range. This agrees with previous reports of very high density of CB1 receptors in brain (Herkenham et al., 1991b; Jansen et al., 1992), which occur in at least 10-fold higher density than other known G-protein-coupled receptors in brain (Sim et al., 1996c).

Table 2 provides apparent KD and Bmax values for net WIN 55212-2-stimulated [35S]GTPγS binding in 10 brain regions. Apparent KD values for [35S]GTPγS binding showed no significant differences between regions (ANOVA, P = 0.37), and had a mean value of 2.8 ± 0.1 nM. In contrast, mean apparent Bmax values differed significantly over a 2.5-fold range (ANOVA, P < 0.0001). The highest level of binding was measured in the hypothalamus, with an apparent Bmax of 18.5 ± 0.5 pmol/mg of activated G-protein, and the lowest level of [35S]GTPγS binding was measured in sensormotor cortex membranes, which had only 7.5 ± 1.5 pmol/mg. The numbers of activated G-proteins did not vary greatly across regions, but it is interesting to note that there were large numbers of activated G-proteins in every region measured, in agreement with the large number of cannabinoid receptors found in rat brain. Furthermore, activated G-protein densities did not vary across regions in proportion to the receptor densities as discussed below.

In general, [3H]SR141716A and [3H]WIN 55212-2 receptor binding Bmax values significantly correlated with each other across regions with r = 0.89 (data not shown). The differences between Bmax values for 3H-labeled antagonist and 3H-labeled agonist binding reflect differences in the fractions of high-affinity agonist binding, as described below. In contrast, net WIN 55212-2-stimulated [35S]GTPγS binding Bmax values did not correlate with the respective regional receptor Bmax values for [3H]SR141716A or [3H]WIN 55212-2 binding, with r = 0.23 and 0.56, respectively. This lack of correlation between receptors and activated G-proteins, in agreement with the autoradiographic data, indicated that the calculated receptor/transducer amplification factors for some brain regions were significantly different from others.

Receptor/transducer amplification factors and fractions of high-affinity agonist binding. Receptor/transducer amplification factors have been defined as the ratio of the apparent Bmax of maximal receptor-stimulated [35S]GTPγS binding to receptor Bmax, and reflects the relative number of G-proteins activated per receptor under receptor saturating conditions. Values for all regions are presented in figure 3. These ratios were calculated in two different ways. The total amplification factor (fig. 3A) was calculated from [3H]SR141716A binding Bmax values and accounts for the total number of (coupled and uncoupled) receptor sites. The coupled amplification factor (fig. 3B) was calculated from high-affinity [3H]WIN 55212-2 receptor binding, and thus only considers high-affinity agonist binding (coupled) sites.

Total amplification factor values (fig. 3A) ranged from approximately 2.0 ± 0.2 in the frontal cortex to 7.5 ± 1.1 in the hypothalamus. The 10 regions were arranged into three groups with low, moderate and high total amplification factors; each group differed significantly from the others. The regions exhibiting low amplification factors (2.0 ± 0.2 to
mean values G-protein-coupled cannabinoid receptors. All data shown represent each region, determined by high-affinity [3H]WIN 55212–2 binding to each region. Panel B depicts coupled amplification factor values for [3H]SR141716A (antagonist)-binding cannabinoid receptors within a given region reflect the G-protein coupling state of the receptors present, because high-affinity [3H]WIN 55212–2 agonist) binding requires receptor/G-protein coupling (Devane et al., 1988), whereas [3H]SR141716A (antagonist) binding does not (Rinaldi-Carmona et al., 1996). The fraction of high-affinity agonist binding to total receptor binding ranged from 0.45 ± 0.1 in the thalamus to 1.4 ± 0.2 in the hypothalamus (fig. 4).

The differences in receptor $B_{\text{max}}$ values for the two ligands within a given region reflect the G-protein coupling state of the receptors present. Fractions of high-affinity agonist binding to cannabinoid receptors were not significantly different from [3H]SR141716A $B_{\text{max}}$ values only in the thalamus and colliculi. This indicated that each of these regions had a significant fraction of uncoupled receptors, even under assay conditions that favored receptor/G-protein coupling (absence of sodium and GDP). The receptor $B_{\text{max}}$ values from the remaining regions were not significantly different between the two ligands (P > .10), which indicated that these regions had relatively few uncoupled receptors.

**Relationship of receptor/transducer amplification factors to receptor occupancy.** The previous determinations of amplification factors were made with a concentration of the agonist WIN 55212–2 (3 μM) that resulted in near-complete receptor occupancy and maximal stimulation of $[^{35}\text{S}]$GTP$\gamma$S binding. To determine whether receptor/transducer amplification factors were different at lower levels of receptor occupancy, three representative regions were as-

Coupled amplification factor values (fig. 3B) ranged from 2.3 ± 0.2 in the hippocampus to 6.8 ± 0.7 in the thalamus. The 10 regions were again arranged by significant differences into low, moderate and high coupled amplification factors. The same four regions that displayed low total amplification factors (frontal cortex, cerebellum, hippocampus and striatum) exhibited low coupled amplification factors (2.3 ± 0.1 to 2.6 ± 0.2). The regions that displayed moderate coupled amplification factors (3.1 ± 0.6 to 4.5 ± 0.4) included the brainstem, amygdala and sensomotor cortex. Sensomotor cortex again had a moderate value (3.1 ± 0.6) which was different from the high, but not from the low amplification factor regions. The thalamus, colliculi and hypothalamus had the highest coupled amplification factors (5.3 ± 0.1 to 6.8 ± 0.7).

The 10 regions were again arranged by significant differences into low, moderate and high coupled amplification factor groups. By Student’s $t$-test, $P < .005$ for regions marked with different letters. Abbreviations for regions are as follows: Amyg, amygdala; BS, brainstem; Cblm, cerebellum; Coll, colliculi; Fr Ctx, frontal cortex; Hippo, hippocampus; Hypo, hypothalamus; S-M Ctx, sensomotor cortex; Thal, thalamus.

2.5 ± 0.3) were the frontal cortex, cerebellum, hippocampus and striatum. The regions displaying moderate amplification factors (3.1 ± 0.5 to 4.5 ± 0.7) included the thalamus, colliculi, brainstem, amygdala and sensomotor cortex. Although the sensomotor cortex exhibited a moderate amplification factor (2.9 ± 0.6) its value did not differ significantly from the values for hippocampus or striatum, which displayed low amplification. Hypothalamus had the highest total amplification factor, and it was significantly greater than those for all other regions.

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The differences in receptor $B_{\text{max}}$ values for the two ligands within a given region reflect the G-protein coupling state of the receptors present, because high-affinity [3H]WIN 55212–2 (agonist) binding requires receptor/G-protein coupling (Devane et al., 1988), whereas [3H]SR141716A (antagonist) binding does not (Rinaldi-Carmona et al., 1996). The fraction of high-affinity agonist binding to total receptor binding ranged from 0.45 ± 0.1 in the thalamus to 1.4 ± 0.2 in the hypothalamus (fig. 4). Statistical significance was determined by testing whether the mean [3H]WIN 55212–2 $B_{\text{max}}$ was equal to the mean [3H]SR141716A $B_{\text{max}}$ by the Student’s $t$-test at $P < .05$. For each region, [3H]WIN 55212–2 $B_{\text{max}}$ values were less than or not significantly different from [3H]SR141716A $B_{\text{max}}$ values. [3H]WIN 55212–2 $B_{\text{max}}$ values were significantly lower than the [3H]SR141716A $B_{\text{max}}$ values only in the thalamus and colliculi. This indicated that each of these regions had a significant fraction of uncoupled receptors, even under assay conditions that favored receptor/G-protein coupling (absence of sodium and GDP). The receptor $B_{\text{max}}$ values from the remaining regions were not significantly different between the two ligands ($P > .10$), which indicated that these regions had relatively few uncoupled receptors.

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sayed with various concentrations of WIN 55212–2 in the presence and absence of 2 nM SR141716A, and for receptor binding with the same concentrations of WIN 55212–2 under \([^{35}\text{S}]\text{GTP}\gamma\text{S}\) binding conditions (with sodium and GDP). Because \(^{3}\text{H}\)-labeled agonist binding cannot practically be conducted under \([^{35}\text{S}]\text{GTP}\gamma\text{S}\) binding conditions, WIN 55212–2 displacement of \([^{3}\text{H}]\text{SR141716A}\) was measured. The cerebellum, amygdala and hypothalamus were chosen because they represent the full range of measured receptor densities and amplification factors (see table 1 and fig. 3). The cerebellum is among the highest regions for receptor density, but lowest for amplification; the hypothalamus exhibits low receptor density and the highest amplification; and the amygdala has intermediate values.

The parameters of WIN 55212–2 stimulation of \([^{35}\text{S}]\text{GTP}\gamma\text{S}\) binding and displacement of \([^{3}\text{H}]\text{SR141716A}\) receptor binding are shown in table 3. The \(ED_{50}\) value for WIN 55212–2 stimulation of \([^{35}\text{S}]\text{GTP}\gamma\text{S}\) binding in cerebellum was significantly higher than the corresponding value for the amygdala \((P < .05)\), but not the hypothalamus. The \(K_{i}\) value for WIN 55212–2 displacement of \([^{3}\text{H}]\text{SR141716A}\) binding in cerebellum was significantly higher than the values for both amygdala and hypothalamus \((P < .05)\). These data were used to calculate \(K_{i}/ED_{50}\) ratios, a useful measure of receptor reserve (Kenakin and Morgan, 1989). In the amygdala and hypothalamus the \(K_{i}/ED_{50}\) ratios were not significantly different from one; in contrast, in the cerebellum the \(K_{i}/ED_{50}\) ratio of 2.4 was significantly different from one \((P < .02)\). A \(K_{i}/ED_{50}\) ratio greater than one indicates receptor reserve, so a slight degree of receptor reserve is indicated for the cerebellum.

Figure 5A compares the concentration-effect curves for WIN 55212–2 in occupying receptors and in activating G-proteins in cerebellum. Receptor occupancy was calculated with the \(K_{i}\) of WIN 55212–2 displacement of \([^{3}\text{H}]\text{SR141716A}\) binding, and activated G-proteins were calculated as a fraction of the maximal amount of \([^{35}\text{S}]\text{GTP}\gamma\text{S}\) binding induced by WIN 55212–2 as described in table 3. Because the \(B_{\text{max}}\) values were known for both parameters (tables 1 and 2), binding data could be presented as the total number of binding sites in picomoles per milligram of membrane protein and could therefore be directly compared in the two assay systems. Thus, in the cerebellum (fig. 5A), each concentration of WIN 55212–2 activated a greater number of G-proteins than the number of receptors occupied.

These data were used to determine amplification factors by dividing the number of activated G-proteins by the number of WIN 55212–2-occupied cannabinoid receptors at each concentration of WIN 55212–2. Results showed that although there was variability in the amplification factors at WIN 55212–2 concentrations up to 100 nM, little change in amplification factor was observed from 100 to 10,000 nM WIN 55212–2 in any given region. However, the amplification factor obtained in the cerebellum at 30 nM WIN 55212–2 was significantly different \((P < .05)\) from the amplification factors for cerebellum at 1000 to 10,000 nM WIN 55212–2. Moreover, the differences in regional amplification observed at the \(B_{\text{max}}\) level (fig. 3) were confirmed at lower concentrations of agonist. Thus, amplification factors in hypothalamus were significantly higher
The present study used agonist-stimulated $[^{35}S]$GTPγS autoradiography (Sim et al., 1995) and membrane saturation binding analysis (Gierschik et al., 1991; Traynor and Nahorski, 1995; Sim et al., 1996c) to compare levels of G-protein activation by cannabinoid receptors in different regions of the rat brain. In the autoradiographic analysis, relative levels of G-protein activation and receptor binding were compared on a qualitative basis and provided the rationale for conducting the saturation binding analyses in brain membranes. The basis of this reaction is an agonist-induced increase in the number of binding sites which exhibited high affinity for $[^{35}S]$GTPγS. Thus, the decrease in the apparent $K_D$ of $[^{35}S]$GTPγS binding (from 500–1000 nM to the values in table 2) represents the agonist-induced increase in the probability that a G-protein will bind $[^{35}S]$GTPγS (or GTP) and become activated. However, it is clear that the $[^{35}S]$GTPγS binding assay is more complex than a simple increase in $[^{35}S]$GTPγS affinity. It is also apparent that cannabinoid agonists also decrease the IC$_{50}$ of GDP in inhibiting $[^{35}S]$GTPγS binding, i.e., the agonist lowers the affinity of the activated G-protein for GDP (C.S. Breivogel and S.R. Childers, unpublished observations), a finding we previously reported in the opioid system (Solley et al., 1997). Nevertheless, from a practical point of view, the increase in $[^{35}S]$GTPγS binding that occurs as a result of the increase in $[^{35}S]$GTPγS affinity provides the basis for detecting receptor/G-protein coupling.

Another potential issue in analyzing these data is the question of equilibrium for $[^{35}S]$GTPγS binding. Previous studies have suggested that $[^{35}S]$GTPγS binding to G-proteins is “quasi-irreversible” (Pfeuffer and Helmerreich, 1975). However, in brain membranes with cannabinoid-stimulated $[^{35}S]$GTPγS binding, this does not appear to be the case. Kinetic experiments in cerebellar membranes revealed that cannabinoid-stimulated $[^{35}S]$GTPγS binding associated with a $t_{1/2}$ of approximately 40 min and dissociated (using excess unlabeled GTPγS) with a $t_{1/2}$ of 30 min in the presence of 3 μM WIN 55212–2 and 30 μM GDP (C.S. Breivogel and S.R. Childers, unpublished observations). Thus, although the equilibrium of $[^{35}S]$GTPγS binding depends on many factors including agonist-receptor, receptor-G-protein, G$_{αi}$βγ, G$_{αs}$ GDP and G$_{αi}$($[^{35}S]$GTPγS interactions, it may be subject to equilibrium binding data if conducted under appropriate and defined assay conditions. It should be noted that absolute binding parameters for $[^{35}S]$GTPγS binding depend on the concentration of GDP. Because 30 μM GDP was used in these studies, the terms “apparent $K_D$” and “apparent $B_{max}$” refer to saturation binding parameters obtained in $[^{35}S]$GTPγS binding assays.

The ratio of apparent $B_{max}$ values from WIN 55212–2-stimulated $[^{35}S]$GTPγS binding and $B_{max}$ values of cannabinoid receptor binding yields receptor/G-protein amplification factors, which can be interpreted as a relative measure of the number of G-proteins that a cannabinoid receptor is able to activate. It should be noted that these amplification factors are not absolute and may be higher under physiological conditions in intact systems. The $[^{35}S]$GTPγS binding assay may underestimate physiological amplification for two reasons. First, there are differences in the binding of GTPγS, a slowly hydrolyzable GTP analog, compared with GTP. In intact cells, GTP is the substrate for the activated G-protein, which hydrolyzes the GTP to GDP to become inactivated. Because $[^{35}S]$GTPγS is so poorly hydrolyzed, the G-protein is unable to cycle and become activated more than once. Even if the G-protein were able to cycle, this technique measures only the $[^{35}S]$GTPγS bound at the termination of the assay. Second, it is not clear what proportion of the ligand-binding receptors are involved in the activation of G-proteins. However, it appears that maximal WIN 55212–2-stimulated $[^{35}S]$GTPγS binding requires full occupancy of membrane cannabinoid receptors, because the G-protein activation and receptor occupancy curves for WIN 55212–2 are parallel and exhibit similar ED$_{50}$ and $K_I$ values, respectively.

With either autoradiography or membrane saturation binding analysis, there were many significant differences in regional amplification factor values. From autoradiographic analysis, the relatively few cannabinoid receptors in the amygdala and hypothalamus appeared to activate nearly as many G-proteins as in the receptor-dense hippocampus or entopeduncular nucleus, and more G-proteins than in the thalamus or cortex, which had intermediate receptor densities. Membrane assay saturation binding analyses yielded total ($[^{3}H]$-labeled antagonist) amplification factor values that varied over nearly a 4-fold range from 2.0 to 7.5 G-proteins activated per receptor. Coupled ($[^{3}H]$-labeled agonist) amplification factor values varied over a 3-fold range from 2.3 to 6.8 G-proteins per receptor. Moreover, the fractions of high-affinity agonist binding in 2 of the 10 regions assayed were significantly less than one, and thus had a significant fraction of receptors that remained uncoupled from G-proteins under assay conditions that favored such coupling.

Although there were many significant differences in amplification factors among different brain regions, the values for cannabinoid receptors were all substantially lower than those previously calculated for mu and delta opioid receptors in rat striatum. In the previous study, striatal opioid receptors activated approximately 20 G-proteins per agonist-binding receptor (Sim et al., 1996c), whereas in both the previous and present studies agonist-binding cannabinoid receptors activated between 2 and 7.5 G-proteins. This demonstrates that cannabinoid receptors in brain couple with relatively low efficiency to G-proteins when compared with opioid receptors.

In previous studies of the cannabinoid receptor system (Sim et al., 1996c), amplification factors were calculated by agonist binding to determine receptor numbers. High-affinity binding of $[^{3}H]$WIN 55212–2 is sensitive to guanine nucleotides, Na$^+$ and Mg$^{2+}$, which indicates that it is dependent on coupling of cannabinoid receptors to G-proteins (Devane et al., 1988), and therefore only measures those receptors that are coupled to a G-protein under the conditions of the agonist binding assay. Such an analysis will usually yield a lower $B_{max}$ (and thus a higher amplification factor value) than when receptor binding is performed with an antagonist. The current study used both an agonist and an antagonist to determine receptor levels in the various brain regions. In some regions, determination of receptor density by high-affinity agonist binding was complicated by the appearance of both high (3 nM) and low (>100 nM) affinity $[^{3}H]$WIN
binding sites. The low-affinity agonist sites were most likely uncoupled cannabinoid receptors, because the unlabeled antagonist displaced a high concentration of \([^{3}H]\)WIN 55212–2 (12 nM) with high affinity, and the cannabinoid agonist CP55940 displaced the same concentrations of \([^{3}H]\)WIN 55212–2 with low affinity (data not shown). These findings were consistent with the coupled and uncoupled agonist affinity states traditionally observed with G-protein-coupled receptors. Although \([^{3}H]\)WIN 55212–2 binding analysis was conducted on both one- and two-site fits by LIGAND, this low-affinity site was responsible for a higher degree of variability in calculated high-affinity agonist binding values of some regions. For example the presence of a relatively large proportion of low-affinity sites was probably the reason that the \(K_{D}\) of \([^{3}H]\)WIN 55212–2 in the colliculi was twice that of any other region. In contrast, the binding of the antagonist, \([^{3}H]SR141716A\), is not sensitive to either guanine nucleotides or sodium (Rinaldi-Carmona et al., 1996), and only one site was detected in all regions, even in the presence of 100 mM NaCl and 30 \(\mu\)M GDP. Antagonist binding therefore measures receptor \(B_{\text{max}}\) values more reliably than agonist binding. Furthermore, antagonist receptor binding has the advantage of being more readily comparable with, and more relevant to, agonist-stimulated \([^{35}S]\)GTP\(\gamma\)S binding. The presence of GDP and sodium in both the \([^{35}S]\)GTP\(\gamma\)S and \([^{3}H]SR141716A\) binding assays induces cannabinoid receptors to a low-affinity state for agonist (WIN 55212–2). In contrast, \(^{3}H\)-labeled agonist binding is performed in the absence of GDP and sodium to provide high-affinity agonist binding to cannabinoid receptors, which is not present under \([^{35}S]\)GTP\(\gamma\)S binding conditions. Because the concentration of WIN 55212–2 used to stimulate \([^{35}S]\)GTP\(\gamma\)S binding is sufficient to saturate all high- and low-affinity cannabinoid receptors, \(^{3}H\)-labeled antagonist measurements of receptor numbers are more relevant to the activation of G-proteins measured in the \([^{35}S]\)GTP\(\gamma\)S binding assay.

The finding of differences in amplification factors across regions was observed in both membranes and by in vitro autoradiography of brain sections. However, some quantitative differences were also observed which were probably caused by the differences in anatomical resolution of the two methods. Small nuclei with high levels of cannabinoid receptors were differentially included in dissected brain regions. For example, the thalamus membrane preparation contained tissue from the entopeduncular nucleus, the brainstem contained substantia nigra and the striatum included globus pallidus. Moreover, such dissections inevitably lose the spatial arrangement (e.g., rostral-caudal differences) that are clearly demonstrated by autoradiography. Thus, although membrane saturation binding analysis has the advantage of being more quantifiable, the fine anatomical resolution of autoradiographic analysis was unattainable by regional dissection.

As defined in this study, amplification factors were calculated at saturating concentrations of agonist. However, in brain it is difficult to predict how often cannabinoid receptors will be saturated with agonist. For this reason, it was of interest to calculate amplification factors at varying receptor occupancy. Figure 5 shows that there was little effect of agonist concentration (i.e., receptor occupancy) on the catalytic amplification factors between 100 and 10,000 nM WIN 55212–2. The amplification factor was difficult to calculate precisely at low agonist concentrations (<100 nM) because of the low values for both activated G-proteins and occupied receptors. Nevertheless, there was a significant increase in amplification factor observed in cerebellum at the lowest concentration of WIN 55212–2 used, 30 nM. It was interesting that in the three regions examined for \(K_{D}/E_{50}\) ratios, only the cannabinoid receptor-rich cerebellum had a ratio greater than one (implying a slight receptor reserve), whereas the intermediate and low receptor density regions, amygdala and hypothalamus, had \(K_{D}/E_{50}\) ratios equal to one. The slight receptor reserve in cerebellum predicts the increase in amplification factor at low concentrations of WIN 55212–2 (fig. 5B): because of the existence of receptor reserve, low receptor occupancy results in a relatively higher level of transducer activation. Furthermore, as the activation of transducer becomes maximal and levels off with increasing agonist concentration, receptor occupancy continues to increase which results in a decreasing ratio of activated G-proteins to occupied receptors. If any G-protein-coupled receptor system in rat brain were to exhibit reserve for the activation of G-proteins, it might be the cannabinoid system, because there is a large excess of cannabinoid receptors compared with other known G-protein-coupled receptors (Herkenham et al., 1991b; Sim et al., 1996a). The fact that there was only slight receptor reserve observed in cerebellum, and none in the other regions, is probably because of the great excess of G-proteins in rat brain (Asano et al., 1990) even when compared with the number of CB1 receptors (table 1). The fact that some reserve was measured in a region with high receptor density agrees well with results in the mu opioid system: \(K_{D}/E_{50}\) ratios were higher in a mu receptor-transfected cell line with high receptor density (approximately equal to cerebellar CB1 density) than in brain membranes with lower mu receptor density (D. E. Selley and S. R. Childers, unpublished observations).

The mechanisms underlying the differences in receptor/G-protein amplification factors are not yet clear, and will be the focus of future studies. One possibility, as discussed above, is that cannabinoid receptor subtypes (e.g., the splice variants, CB1 and CB1A) may exhibit different levels of catalytic activity. Thus, the regional variation in amplification factors may reflect differences in the ratio of these (and perhaps yet undiscovered) subtypes. Alternatively, the regional differences in amplification factors may in part be caused by the co-localization of the different subtypes of G-protein \(\alpha\) subunits with cannabinoid receptors. Subtypes of \(G_{\alpha1}\) \(\alpha\) subunits (\(G_{\alpha11}, G_{\alpha12}, G_{\alpha13}, G_{\alpha14}\), and \(G_{\alpha12}\)) (Jones and Reed, 1987; Hsu et al., 1990) may be activated specifically or with varying degrees of efficiency by cannabinoid receptors, as seen in other receptor systems (McKenzie and Milligan, 1990; Senogles et al., 1990). If CB1 receptors activate different G-protein \(\alpha\) subunits with varying efficiency, then agonist concentration might affect the composition of the activated subtypes. For instance, low receptor occupancy could preferentially activate one subtype, and additional subtype(s) could be recruited at higher concentrations of agonist. Similarly, G-protein \(\beta\gamma\) subunits have been shown to confer receptor specificity or selectivity of G-protein coupling even when paired with the same \(\alpha\) subunit subtype (Kleuss et al., 1992). Thus, regional amplification factors could vary with the composition of G-protein subunit subtypes present in each re-
gion. However, the similarity of the regional apparent \(K_D\) values for agonist-stimulated \([\text{35S}]\text{GTP}\gamma S\) binding indicate that cannabinoid receptors are activating the same class of G-protein \(\alpha\) subunits \(G_\alpha\) as opposed to \(G_\beta\gamma\) or other subtypes because, for example, \(G_\alpha^{\text{n}}\) binds GTP analogs with much greater affinity than \(G_i\) or \(G_\alpha\) (Rasenick and Childers, 1989). This also agrees with previous reports of cannabinoids acting via pertussis toxin-sensitive \((G_i/o\text{-mediated})\) mechanisms (Childers and Deadwyler, 1996).

Another factor that might contribute to the regional variation of amplification factors is the ratio of receptors to G-proteins. This model is represented by the law of mass action: the higher the concentration of available G-proteins, the more frequently a receptor and G-protein will collide in an interaction that results in the activation of the G-protein. The observation that the highest amplification factors occur in the lower receptor density regions, and that the high-density regions all have low amplification factors supports this hypothesis.

Another explanation is that amplification factors would depend on the degree of cannabinoid receptor/G-protein pre-coupling. That possibility was investigated in the present study by correlating amplification factors with the fractions of high-affinity agonist binding. These data (correlated from figs. 3 and 4) showed that the fraction of high-affinity binding correlated poorly with both coupled amplification factors \((r = 0.3)\) and total amplification factors \((r = 0.6)\) (data not shown). Therefore, the fraction of high-affinity binding is not an important determinant of amplification factor values.

Regional differences in receptor/G-protein amplification factors may help to elucidate the physiological significance of the endogenous cannabinoid system. For example, the hippocampus has high levels of both cannabinoid-activated G-proteins and cannabinoid receptors. Thus, the effects of cannabinoids on short-term memory, which appear to be mediated by the hippocampus (Deadwyler et al., 1995), might be predicted based on the high receptor density. This is in contrast to the hypothalamus, a region with a high level of cannabinoid-activated G-proteins despite low cannabinoid receptor density. The well-established effects of cannabinoids on basal body temperature and hypothalamic hormone function (Dewey 1986; Holister 1986) would not necessarily be predicted based on the relatively low density of cannabinoid receptors in the hypothalamus. Thus, in this region, receptor activation of G-proteins may be a better predictor of cannabinoid efficacy than cannabinoid receptor levels. Therefore, predictions of the magnitude of a drug effect in a given brain region must be made not only on the basis of receptor binding analysis, but also on the degree of activation of intracellular signal transduction mechanisms by those receptors.

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References


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