Characterization of CB₁ Cannabinoid Receptors Using Receptor Peptide Fragments and Site-Directed Antibodies

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ABSTRACT

The mechanism by which CB₁ cannabinoid receptors are coupled to the Gᵢ/Gₒ class of G proteins was studied. A peptide representing the juxtamembrane carboxyl terminus robustly stimulated guanosine-5’-O-(3-thio)triphosphate binding. Peptides simulating subdomains of the third intracellular loop (IL3) activated minimally when present alone but produced additive effects when present in combination. Peptides representing the amino-side IL3 and the juxtamembrane carboxyl terminus autonomously inhibited adenylate cyclase, and this response was not significantly augmented or inhibited by peptides representing other intracellular domains. Site-directed antipeptide antibodies developed against the domains of the amino terminus, first extracellular loop, amino-side IL3, and juxtamembrane carboxyl terminus of CB₁ receptors failed to influence binding of [³H]CP-55940. However, IgG raised against the amino-side IL3 diminished the agonist-dependent inhibition of adenylate cyclase. These experiments suggest that the juxtamembrane carboxyl terminus is critical for G protein activation by CB₁ cannabinoid receptors and that the amino-side IL3 also may interact with Gᵢ proteins leading to inhibition of adenylate cyclase.

Δ⁹-Tetrahydrocannabinol, the active compound in Cannabis sativa, and synthetic cannabimimetic compounds of cannabinoid, aminoalkylindole, and eicosanoid classes interact with cannabinoid receptors in the nervous system to produce effects (for a review, see Howlett, 1995a). Stimulation of CB₁ cannabinoid receptors results in the pertussis toxin-sensitive inhibition of forskolin- or receptor-stimulated adenylate cyclase and regulation of ion channels, indicating that the cannabinoid receptor is coupled to Gᵢ and Gₒ proteins (for a review, see Howlett, 1995b). The sequence of CB₁ cannabinoid receptors has been deduced for both rat and human species (Matsuda et al., 1990; Gerard et al., 1991).

In the current study, we sought to determine topographic regions of the CB₁ cannabinoid receptor that would be critical regions for agonist-induced activity. Peptides representing regions of the IL3 or the juxtamembrane carboxyl terminus were tested for their ability to compete for receptor/G protein interactions or to act autonomously to stimulate G proteins. Site-directed antibodies were generated against synthetic peptides representing the amino terminus, EL₁, amino-side segment of the IL3, and juxtamembrane carboxyl-terminal regions of the rat brain CB₁ receptors. These antisera were tested for their ability to occlude ligand binding to the receptor or coupling of the receptor to G proteins in membrane preparations. Based on the findings of the peptide and antibody interactions, it was concluded that the juxtamembrane carboxyl terminus is critical for G protein activation by CB₁ cannabinoid receptors and that the amino-side IL3 also may interact with Gᵢ proteins leading to inhibition of adenylate cyclase.

Materials and Methods

Peptides. Peptides (Table 1) CB₁–14, CB₁74–188, CB₃₀₁–₃₁₇, and CB₄₀₁–₄₁₇ were synthesized using t-butyloxycarbonyl chemistry and purified by C-18 reverse-phase high performance liquid chromatography eluted with a gradient extending over 35 min from 0.1% trifluoroacetic acid to 80% acetonitrile plus 0.085% trifluoroacetic acid. Peptides CB₃₁₆–₃₂₇, CB₃₂₉–₃₄₄, and CB₄₀₁–₄₁₇ were synthesized by 9-fluorenylmethoxycarbonyl chemistry and purified by C-18 reverse-phase high performance liquid chromatography (Princeton Biomolecules, Columbus, OH). The amino acid sequence of peptide CB₄₀₁–₄₁₇ was verified by amino acid sequence analysis using automated Edman degradation and gas chromatography/mass spectrometry. There is 100% identity in the amino acid sequences of these six peptides between rat and human CB₁ receptors (Matsuda et al., 1990; Gerard et al., 1991). CB₁–14 is 64 amino acids proximal to the first of three potential amino-linked glycosylation sites on the CB₁ cannabinoid receptor.

ABBREVIATIONS: IL3, third intracellular loop of the G protein-coupled receptor; BSA, bovine serum albumin; CHAPS, 3-{[3-cholamidopropyl]-dimethylammonio}propanesulfonate; DALN, desacyllevonantradol; EL₁, first extracellular loop of the G protein-coupled receptor; ELISA, enzyme-linked immunosorbent assay; GTP-γ-S, guanosine-5’-O-(3-thio)triphosphate; KLH, keyhole limpet hemocyanin.
amino terminus, and therefore the epitope on the receptor is not likely to be obscured by the bulky oligosaccharides. The peptides have poor homology (0–30%) with related domains of the CB1 receptor, with the exception of CB174–188 (EL1), which exhibits 64.3% identity and 5 consecutive amino acids.

**Antibodies and immunobLOTS.** Peptide CB1–14, modified by a cysteine at the carboxyl terminus (CB1–14), was conjugated at a 40-fold molar excess to sulfo-SMCC-KLH (Pierce Chemical, Rockford, IL). CB301–317 and lysine-modified CB174–188 and CB401–417 were conjugated to KLH in an 8–40-fold molar excess of peptides using glutaraldehyde (Song and Howlett, 1995). In CB401–417, Cys416 was replaced with serine to avoid potential artifactual disulfide bond formation. New Zealand White rabbits were injected intradermally with peptide-KLH conjugates (50 μg/rabbit), and antisera were screened by ELISA using peptide-BSA conjugates on Immulon 2 plates (Dynatech, Chantilly, VA). Plates were incubated and washed sequentially with antisera, goat anti-rabbit IgG conjugated to horseradish peroxidase (Pierce) (1:6,000), and 2,2-azino-di-[3-ethylbenzthiazoline)-6 sulfonate (Pierce) in 2.5 mM H2O2, 0.1%Tween 20, and 100 mM sodium acetate, pH 4.2, and the absorbance at 405 nm was determined with a Molecular Devices (Menlo Park, CA) plate reader. By ELISA, all antisera recognized their respective immunization peptides in the peptide-BSA conjugates but not unconjugated BSA or alternative peptide conjugates; the corresponding preimmune sera showed no significant reaction. Titers achieved were 0.16–0.21 mg/ml. The hormone-stimulated enzyme activities were 0.16–0.21 mg/ml leupeptin, 50 μg/ml soybean trypsin inhibitor, 5 μg/ml pepstatin). Rat brain phosphodiesterase inhibitor. Final protein concentrations were measured by absorbance at 280 nm.

**Membrane preparation and signal transduction assays.** Rat brain membranes were prepared from entire rat brain minus the brainstem as described previously (Devane et al., 1988) with the addition of a protease inhibitor cocktail to the buffers (15 μg/ml benzamidine, 5 μg/ml leupeptin, 50 μg/ml soybean trypsin inhibitor, 1 mM phenylmethylsulfonyl fluoride, and 0.7 μg/ml pepstatin). N18TG2 cell membranes were prepared as described previously (Howlett, 1985).

Radioligand binding assay of the CB1 cannabinoid receptor was performed and analyzed using [35S]GTPγS as described previously (Pinto et al., 1994), with specific binding defined as [35S]GTPγS binding that was displaced by 100 nM DALN. [35S]GTPγS binding to rat brain membrane G proteins was performed according to a modification of the procedure of Lorenzen et al. (1993). Rat brain P2 membranes (5 μg of protein) were added to a reaction mixture containing 50 mM Tris-Cl, pH 7.5, 1 mM EDTA, 5 mM MgCl2, 100 mM NaCl, 1 mM diethiolethiol, 10 μM GDP, 0.375 mM [35S]GTPγS, and the indicated concentrations of peptides or cannabinoid ligands (100 μM final volume). Binding of [35S]GTPγS (30’ for 60 min) was stopped by rapid filtration over GF/B (Whatman, Clifton, NJ) filters using a TOMTEC (Orange, CT) Harvester, and radioactivity was quantified using a 1205 Betaplate counter (Wallac, Gaithersburg, MD). Nonspecific binding was calculated as binding that could not be displaced by 100 μM GTPγS. Data were calculated as pmol of [35S]GTPγS bound/mg of protein from an average of four replicates. Background values averaged 623 ± 68 fmol/mg (mean ± standard error, six experiments), and values stimulated by 1 μM DALN averaged 1150 ± 100 fmol/mg (six experiments).

**Results**

**Determination of Functional Domains on Rat Brain Cannabinoid Receptors**

**Agonist binding sites.** All study antisera specifically recognized a band with an apparent molecular mass of 64 ± 1 kDa (13 experiments), whereas preimmune sera did not (Song and Howlett, 1995; data not shown). The 64-kDa band is believed to be CB1 cannabinoid receptors with carbohydrates at two glycosylation sites (Song and Howlett, 1995). To determine whether the domains of the receptor occluded by the antibodies overlap regions important for agonist ligand binding, rat brain membrane preparations were preincubated with each of the four antisera, and specific binding of [3H]CP-55940 was determined. Compared with the effects of cognate preimmune sera, none of the antisera interfered with ligand binding to rat brain cannabinoid receptors (Table 2).

The more concentrated antisera and preimmune sera (1:20 dilution) both attenuated the specific binding of [3H]CP-55940, probably as the result of an interaction of serum lipoproteins and albumin with radioligand. Serum lipoprotein and albumin interactions with cannabinoid ligands have been reported previously (Wahlqvist et al., 1970; Widman et al., 1974; Poddar et al., 1988) at concentrations equal to or greater than present in the assay mixture (1.2 mg of protein/ml for 1:20, 4.8 mg of protein/ml for 1:5 in Table 3).

**Sites coupling to G proteins.** Receptor peptide fragments and site-directed antibodies were used to investigate domains of the CB1 receptor important for receptor/G protein interaction. None of the receptor peptide fragments (at 100 μM) was able to compete for DALN-induced receptor/G protein interactions as determined in either [35S]GTPγS binding or adenylate cyclase assays (see Figs. 3 and 6). To determine whether receptor peptide fragments could autonomously activate Go, the peptides were added with membranes directly into the incubation mixture in the absence of a stim-
TABLE 2

The effect of site-directed antisera on agonist ligand binding to rat brain cannabimimetic receptors

Rat brain P2 membrane preparations were incubated with no sera, antisera, or preimmune sera at 4° for 4 hr before the addition (without washing) to the ligand binding reaction mixtures at 0.4 times the final assay volume. Under the conditions of the assay, the specific binding activity of 110 pg [35S]CTP-S55940 in the absence of serum was 56.2 ± 4.3 and 61.2 ± 0.6 fmol/mg of protein for experiments A and B, respectively. These values represented ~70% of the total binding at this concentration of radioligand and membrane fraction. The data shown are the mean ± standard error from two experiments, each with triplicate determinations. There was no significant difference (p < 0.05) when the effect of antisera was compared with that of preimmune sera using a paired Student’s t test.

<table>
<thead>
<tr>
<th>Antibody</th>
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<th>Specific binding</th>
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<td></td>
<td></td>
<td>Percent of total binding</td>
<td>Normalized to control</td>
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<tr>
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*Significantly lower than preimmune serum control at p < 0.05 using paired Student’s one-tailed t test.

The response to peptides was additive with agonist-dependent stimulation by DALN (Fig. 3). This might suggest that the agonist/CB1 receptor complex is a limiting factor in G protein stimulation in rat brain homogenates. An explana-

![Fig. 1. Effect of CB1 receptor peptide fragments on [35S]GTPγS binding to rat brain membrane G proteins. Peptides (100 μM) were included in the incubation mixture without pretreatment, and accumulation of [35S]GTPγS was determined. Data are presented as activation promoted by the peptide expressed as a percentage of the response to 1 μM DALN (i.e., stimulated by agonist/receptor complex), where 0% is the [35S]GTPγS binding in the absence of hormonal or peptide stimulators. Error bars, standard error from three to five experiments for each peptide. Data were analyzed by analysis of variance followed by Tukey’s post hoc test, and no significant differences (p < 0.05) were noted between peptides except for CB401–417, which differed from all others.](aspetjournals.org)
tion for the additivity could be that the peptide fragments were activating G proteins that were not associated with agonist/receptor complexes. One could hypothesize that these G proteins would be of the Gi/o class; however, the activation of other G protein classes cannot be dismissed.

Cannabinoid receptor-stimulated [35S]GTPγS binding in rat brain homogenates would be likely to represent predominantly Gs (Garibay et al., 1991). To determine whether peptide fragments of the CB1 receptor could autonomously activate Gs, these peptides were added directly to the adenylate cyclase incubation mixture, and enzyme activity was measured in the absence of an agonist (Fig. 4). The peptides representing the extracellular amino terminus and EL1 produced a minimal response in the adenylate cyclase assays. The juxtamembrane carboxyl terminus robustly promoted inhibition of adenylate cyclase, with an efficacy that generally exceeded that of cannabinoid agonist/receptor-stimulated activity. The amino-side IL3 also inhibited adenylate cyclase, but the extent was limited to only ~40% of that promoted by the agonist/receptor complex. The magnitude of the response was not increased when 300 μM peptide was used (data not shown). The peptide fragments representing the central IL3 and carboxyl-side IL3, even at 300 μM, failed to evoke a response greater than those of the two extracellular domain fragments.

Combinations of peptides were tested to determine whether discrete CB1 receptor domains could interact with each other to promote Gs activation. No synergism was observed on the addition of peptides representing the central and/or carboxyl-side regions of IL3 to the amino-side IL3 peptide (Fig. 5). Peptide combinations comprising IL3 failed to significantly alter the response to the juxtamembrane carboxyl-terminal domain peptide. Thus, the dominant activating effect of the amino-side carboxyl-terminal domain was neither attenuated nor augmented by the presence of any of the subdomains of IL3.

The cannabinoid agonist DALN inhibited adenylate cyclase, but the extent was limited to only ~40% of that promoted by the agonist/receptor complex. The magnitude of the response was not increased when 300 μM peptide was used (data not shown). The peptide fragments representing the central IL3 and carboxyl-side IL3, even at 300 μM, failed to evoke a response greater than those of the two extracellular domain fragments.

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Adenylyl cyclase activity with an EC$_{50}$ value of 31 nM (confidence interval, 16–64 nM) and an asymptotic approach to a maximal inhibition of 40% of the hormone-stimulated activity in these experiments. Peptide CB301–317 alone inhibited adenylyl cyclase activity by 15% and produced a less-than-additive response with increasing concentrations of DALN (Fig. 6). In the presence of peptide CB301–317, the EC$_{50}$ value for DALN was 9.5 nM (confidence interval, 2–47 nM) and approached a maximal inhibition of 40% of the hormone-stimulated activity. Thus, there seemed to be a limitation in the amount of G$_i$ activated in the presence of this peptide, and the response to agonist/receptor complex seemed to supersede that of the receptor fragment.

Peptide CB401–417 alone produced a robust inhibition of adenylyl cyclase (32%) at 100 µM, a concentration that produced the maximum response (Mukhopadhyah S and Howlett AC, unpublished observations). This concentration of peptide CB401–417 produced additive effects with the agonist/receptor complex throughout the entire dose-response range for the agonist. In the presence of CB401–417, the EC$_{50}$ value for DALN was 19 nM (confidence interval, 4–90 nM) and approached a maximal inhibition of 65% of the hormone-stimulated activity. Analysis of the curves indicates that the small decreases in EC$_{50}$ values in the presence of peptides are not statistically significant. The increase in efficacy produced by peptide CB401–417 suggests a greater number of G$_i$ proteins are activated in the presence of the receptor peptide fragment than are activated by the agonist/receptor complex alone. This suggests that the population of G$_i$ proteins available for interaction with the peptide is distinct from the population of G$_i$ proteins coupled to CB$_1$ receptors. The observations that the actions of hormone-stimulated receptor and peptide CB401–417 are neither synergistic nor competitive, and that the peptide did not alter the affinity of the hormone for the receptor suggests the peptide does not affect receptor/G protein coupling.

Agonist-induced inhibition of adenylyl cyclase was assayed in N18TG2 membranes that had been preincubated with each of the four site-directed antisera or preimmune sera. Anti-CB1–14 and anti-CB174–188, antibodies that would occlude the amino terminus and EL1 of CB$_1$ receptors, each failed to alter the DALN-induced inhibition compared with the effect of the cognate preimmune sera (Table 3). Preincubation of membranes with antiserum against IL3 (anti-CB301–317) resulted in a dose-dependent attenuation of the DALN-induced inhibition of adenylyl cyclase compared with the effect of cognate preimmune serum. The antiserum against the amino-side carboxyl terminus (anti-CB401–417) did not inhibit the effect of DALN on adenylyl cyclase. The influence of the preimmune sera at low dilutions suggests interferences that might be eliminated by isolating the IgG fraction by Protein A chromatography. The normalized DALN-induced inhibition of adenylyl cyclase in the presence of IgG fractions of anti-CB301–317 versus cognate preimmune serum was 68.4% versus 93.2% at a low concentration of IgG (0.016 mg/ml) and 50.5% versus 85.5% at a high concentration (0.163 mg/ml) (Fig. 7). The IgG of anti-CB401–417, against the amino-side carboxyl terminus, failed to block the DALN response (Fig. 7). These data suggest that the CB$_1$ receptor IL3 may be involved in G$_i$/receptor coupling. However, the lack of response to the IgG of anti-CB401–417 cannot necessarily be interpreted to mean that this region fails to be involved in G$_i$/receptor coupling. The lack of response may simply be due to a poor affinity that the antibody may have for the receptor, resulting in the inability of the antibody to compete with a receptor/G protein interaction with relatively greater affinity.

### Discussion

In the current report, we describe peptide fragments of the CB$_1$ cannabinoid receptor and four site-directed anti-peptide antisera against CB$_1$ receptors and the use of these tools to study functional domains of brain cannabinoid receptors. An important finding from these studies is that the juxtamembrane carboxyl-terminal domain peptide of the CB$_1$ cannabinoid receptor is a highly effective activator of G$_i/o$ proteins. Interaction of this receptor domain with G proteins has been reported for several other G protein-coupled receptors, including rhodopsin coupling to transducin as indicated by studies using synthetic peptides (Takemoto et al., 1985; Phillips and Cerione, 1994) and alanine scanning mutagenesis (Osawa and Weiss, 1994). The amino-side carboxyl-terminal

![Fig. 6. Additivity between activating peptides and agonist/receptor complex for G activation. Peptides CB301–317 and CB401–417 (100 µM) were included in the incubation mixture in the presence or absence of increasing concentrations of the cannabinoid agonist DALN. Data are mean ± standard error from three experiments, and the curves were analyzed using GraphPAD InPlot.](image)

![Fig. 7. DALN-induced inhibition of adenylyl cyclase in the presence of IgG fractions of anti-CB301–317 and anti-CB401–417. The IgG fractions derived from the two antisera or their respective preimmune sera were preincubated with the N18TG2 cell membrane at 4°C for 4 hr before direct addition to the adenylyl cyclase assay mixture at 0.4 times the final assay volume. The inhibition of CAMP synthesis by DALN in the absence of any serum was 44%, and this value was normalized to be 100%. Error bars, mean ± standard error from triplicate determinations. Data shown are representative of four experiments.](image)
region seemed to be important for β2-adrenergic receptor coupling to G proteins in mutation studies (O’Dowd et al., 1988). A juxtamembrane carboxyl-terminal peptide from the N-formyl peptide receptor interacted with a carboxyl-terminal region of αi in ELISA studies, and that peptide also disrupted the receptor/G protein complex in detergent solution (Schreiber et al., 1994). A major difference between the results reported here for the CB1 receptor compared with other G protein-coupled receptors is that activation of a G protein by the carboxyl terminus has not yet been reported for other receptor types.

The CB1 receptor proximal amino-side IL3 peptide is able to stimulate Gi activation, leading to inhibition of adenylate cyclase; however, this peptide does not exhibit as great an efficacy as the juxtamembrane carboxyl-terminal peptide. Another difference between the actions of these two peptide segments is that the amino-side IL3 peptide alone does not promote the activation of G proteins in brain membranes (predominantly Gαi). Perhaps the IL3 receptor region facilitates G protein activation secondarily to or only in combination with a more effective stimulus.

For a number of G protein-coupled receptors, the carboxyl-side IL3 domain has been proposed to serve as a G protein recognition or coupling region (Dixon et al., 1987; O’Dowd et al., 1988; Wade et al., 1994; Yamada et al., 1994; Liu et al., 1995; Shi et al., 1995), although the data presented here do not support this role for the carboxyl-side IL3 of the CB1 receptor. Evidence exists for a role for the carboxyl-side IL3 domain of G protein-coupled receptors in suppressing or restricting the G protein interaction in that point mutations in the carboxyl-side IL3 domain of G protein-coupled receptors in suppressing or restricting the G protein interaction in that point mutations in the carboxyl-side IL3 domain of the α1B-adrenergic receptor (Cotecchia et al., 1990; Ren et al., 1993), the α2A-adrenergic receptor (Ren et al., 1993), and muscarinic acetylcholine (Hogger et al., 1995) receptors result in constitutively active receptors. When the carboxyl-side IL3 peptide of the CB1 receptor was included in combination with the peptides that activate Gαi and Gαs, it failed to suppress activation. This evidence argues against a model in which this domain of the CB1 receptor would suppress activity by associating with and thereby occluding a domain that activates G proteins.

For the CB1 receptor, the combination of subdomains of IL3 seems to be necessary to observe activation of G proteins in rat brain membranes. This could result from a summation of the activation of multiple G protein subtypes, each activated by a different receptor domain. Alternatively, a G protein may possess multiple sites of interaction with the receptor, and the combination of peptides may trigger recognition and activation at these distinct sites. The finding that multiple patches on the receptor facilitate interaction with the G protein may have been reported for other G protein-coupled receptors, including the IL2 and IL3 regions of the muscarinic acetylcholine receptor in chimeric mutant studies (Wong et al., 1990); IL2, IL3, and a region of the carboxyl terminus distal to the cysteine-palmitate anchor of rhodopsin in studies of peptides and peptide-directed antisera (Weiss et al., 1988; Konig et al., 1989); IL2, amino-side IL3, and amino-side IL4 of the β2-adrenergic receptor in peptide studies (Munch et al., 1991); and amino- and carboxyl-side IL3 of the α2-adrenergic receptor in studies of homodimers and heterodimers of peptides (Dalman and Neubig, 1991; Wade et al., 1994). Recognition sites on α- and β2-adrenergic and muscarinic acetylcholine receptors have been demonstrated that promote coupling but fail to activate G proteins autonomously (Hausdorff et al., 1990; Luttrel et al., 1993; Hawes et al., 1994). For the β2-adrenergic receptor, deletion mutation studies implicate a seven-amino acid segment in the carboxyl-side IL3 in transmitting the agonist-induced stimulatory signal to Gαi and that this region is distinct from domains that function to promote the ternary complex (Hausdorff et al., 1990). Peptides from the amino-side IL3 and carboxyl-side IL3 of the β2-adrenergic receptor activate Gαi in phospholipid vesicle GTPase and GTP-γS binding assays (Cheung et al., 1991). For the α2-adrenergic receptor, IL2 or the carboxyl-side IL3 peptides reduced high affinity agonist binding, but only the latter could stimulate GTPase activity (Dalman and Neubig, 1991). These findings favor a model of cooperativity between different domains of G protein-coupled receptors to activate G proteins.

The CB1 carboxyl-terminal peptide activates G proteins in a manner that is additive with that of cannabinoid receptor-stimulated G protein activation. The CB1 receptor/G protein complex seems to be stable in the absence of agonist in CHAPS-solubilized preparations (Houston and Howlett, 1993). The peptides seem to be activating a population of G proteins that are not coupled to the cannabinoid receptor. This differs from observations made for several other G protein-coupled receptors for which peptides compete for the receptor/G protein interaction (Konig et al., 1989; Phillips and Cerione, 1994; Schreiber et al., 1994; Wade et al., 1994).

The CB1 receptor/G protein interaction could be disrupted by the antiserum or IgG fraction against the proximal segment of IL3. This finding is consistent with antibody competition for a G protein association site on the receptor. A similar disruption was reported for the D2 receptor, in which an antibody against an IL3 peptide was able to disrupt the ternary complex (Boundy et al., 1993). The incomplete block of the cannabinoid inhibition of adenylate cyclase may suggest that the Gα protein interacts with multiple intracellular domains of the receptor that also may stabilize the receptor/G protein association and that the antibody inefficiently dislodges the G protein.

No effect of the antibody to the juxtamembrane carboxyl-terminal of the CB1 receptor was observed when tested on cannabinoid-mediated inhibition of adenylate cyclase. If the juxtamembrane region of the CB1 receptor association with the G protein is of high affinity, then an antibody may not be able to disrupt that preformed interaction. Alternatively, the CB1 receptor/G protein interaction may occlude the juxtamembrane carboxyl-terminal domain of the receptor. This was the explanation for the failure of a carboxyl-terminal α1B antibody to interact with the α2-adrenergic receptor/G protein ternary complex in the presence of agonist (Okuma and Reisine, 1992).

The current results have provided some insight into the nature of the CB1 receptor/G protein interaction leading to G protein activation and signal transduction. Future studies will investigate the selectivity for G protein subtypes and the role of the α and βγ subunits in the interaction with subdomains of the CB1 receptor. Other studies will investigate the properties of the CB1 receptor carboxyl-terminal juxtamembrane domain that regulate activation of G proteins.
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References


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