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Activation of G-proteins in Brain by Endogenous and Exogenous Cannabinoids

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ABSTRACT

The biological response to cannabinoid agonist begins when the agonist-bound receptor activates G-protein G_α subunits, thus initiating a cascade of signal transduction pathways. For this reason, information about cannabinoid receptors/G-protein coupling is critical to understand both the acute and chronic actions of cannabinoids. This review focuses on these mechanisms, predominantly examining the ability of cannabinoid agonists to activate G-proteins in brain with agonist-stimulated [^{35}S]guanylyl-5′-O-(γ-thio)-triphosphate ([^{35}S]GTPγS) binding. Acute efficacies of cannabinoid agonists at the level of G-protein activation depend not only on the ability of the agonist to induce a high affinity state in G_α for GTP, but also to induce a low affinity for GDP. When several agonists are compared, it is clear that cannabinoid agonists differ considerably in their efficacy. Both WIN 55212-2 and levonantradol are full agonists, while Δ^9-tetrahydrocannabinol is a weak partial agonist. Of interest, anandamide and its stable analog methanandamide are partial agonists. Chronic treatment in vivo with cannabinoids produces significant tolerance to the physiological and behavioral effects of these drugs, and several studies have shown that this is accompanied by a significant loss in the ability of cannabinoid receptors to couple to G-proteins in brain. These effects vary across different brain regions and are usually (but not always) accompanied by loss of cannabinoid receptor binding. Although the relationship between cannabinoid receptor desensitization and tolerance has not yet been established, these mechanisms may represent events that lead to a loss of cannabinoid agonist response and development of tolerance.

KEYWORDS: G-protein, Efficacy, Signal transduction, Anandamide, Delta-9-tetrahydrocannibinol, Levonantradol, Desensitization

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INTRODUCTION

When Howlett first reported the existence of specific cannabinoid receptors,[1] the crucial discovery depended on the fact that cannabinoid agonists inhibited adenylyl cyclase through a G-protein-coupled mechanism. Therefore, it was clear from the beginning that these receptors were members of the G-protein-coupled receptor superfamily. Subsequent studies demonstrated that cannabinoid receptors were indeed coupled to effectors that were modulated by the $G_{i/o}$ class of G-proteins.[2-4] This finding was followed by cannabinoid receptor radioligand binding,[5] receptor localization,[6] and cloning and sequencing of the brain cannabinoid receptor CB_1.[7] The cloning of a peripheral cannabinoid receptor, CB_2, from spleen cells[8] showed that there are at least 2 major types of cannabinoid receptors. Both receptor types are typical of the 7 transmembrane-domain superfamily of receptors, with 44% homology between CB_1 and CB_2 receptors. CB_1 is larger than CB_2, with an additional 72 amino acid residues in the N-terminal region, 15 additional residues in the third extracellular loop, and 13 additional residues in the C-terminal region. The highest degree of homology between CB_1 and CB_2 occurs in the transmembrane regions TM2, TM3, TM5, and TM6; of interest, the homology in other regions is not particularly striking.

From these findings, it is clear that cannabinoid receptors operate by many of the same principles that govern the other receptors in this family of proteins. But cannabinoid receptors also have several properties that make them unique among G-protein-coupled receptors, at least at this stage of our understanding. For example, CB_1 receptors exist in brain at levels higher than most other G-protein-coupled receptors,[5,6] approaching levels observed for amino acid receptors. This fact not only demonstrates the importance of CB_1 receptors in regulating brain activity in a variety of ways, but also has importance in regulating the efficacy of cannabinoid agonists.

Another unique aspect of cannabinoid receptors is the fact that their endogenous ligands represent a class of lipophilic compounds based on the general structure of modified arachidonic acid derivatives. The first of these compounds, arachidonyl ethanolamide or anandamide, was isolated in 1992,[9] followed later by other arachidonyl endogenous cannabinoids including arachidonyl glycerol.[10] Among endogenous agonists at G-protein-coupled receptors, anandamide

is unique in that it is a partial agonist at CB_1 receptors, as will be discussed in detail later.

Acute Effects of Cannabinoids in Activating G-proteins

As observed above, cannabinoid receptors share many of the same properties of G-protein coupling and activation as other members of the GPCR superfamily. For example, guanine nucleotides inhibit cannabinoid agonist binding in a manner typical of G-protein-coupled receptors.[5] Moreover, cannabinoid binding sites can be solubilized from membranes together with G-proteins,[11] and recent evidence suggests that multiple cannabinoid ligands can activate different populations of G-proteins.[12] Cannabinoid receptor activation of G-proteins in isolated membranes can be measured by agonist-stimulated [^{35}S]guanylyl-5′-O-(γ-thio)-triphosphate ([^{35}S]GTPγS) binding.[13] The technique was originally developed in purified systems,[14-16] and in membranes from heart,[17] brain,[18] and cultured cells[19] as a measure of specific receptor-stimulated G-protein activation. An important application for [^{35}S]GTPγS binding is the quantitative estimate of agonist efficacy at the level of G-proteins, first studied with β-adrenergic receptors[20,21] and now applied to a large number of other G-protein-coupled receptors.

Agonist-induced stimulation of [^{35}S]GTPγS binding to G_α is based upon the G-protein activation cycle. The critical step in the [^{35}S]GTPγS assay is addition of excess GDP to shift the G-protein into the inactive state. This step is crucial because spontaneously active G-proteins will bind [^{35}S]GTPγS, increasing the basal level of activity and making agonist-stimulated [^{35}S]GTPγS binding undetectable. After the addition of GDP, [^{35}S]GTPγS and agonist are added to activate those G-proteins coupled to the receptor of interest. Receptor activation decreases the affinity of G_α for GDP and increases its affinity for [^{35}S]GTPγS. In cells, this increase in GTPγS affinity can be 100- to 300-fold.[22] In vivo, the α subunit GTPase hydrolyzes GTP to GDP; however, in vitro, [^{35}S]GTPγS is useful because it is resistant to hydrolysis. Although first developed in isolated membranes, these same principles can be applied (with several technical changes) to brain sections to localize receptor activity in different brain regions. The development of [^{35}S]GTPγS autoradiography represented the first in vitro method to provide a neuroanatomical localization of a receptor-coupled intracellular signal transduction system.[23]

The Scatchard plot in Figure 1 shows the dramatic effect of WIN 55212-2 on activation of G-proteins as measured by [^{35}S]GTPγS binding. This experiment shows the affinities of G_α for [^{35}S]GTPγS in cerebellar membranes in the presence and absence of the agonist. Under basal conditions, in the absence of WIN 55212-2, most of G_α subunits in these membranes display low affinity for [^{35}S]GTPγS, with an equilibrium dissociation constant (K_D) of 540

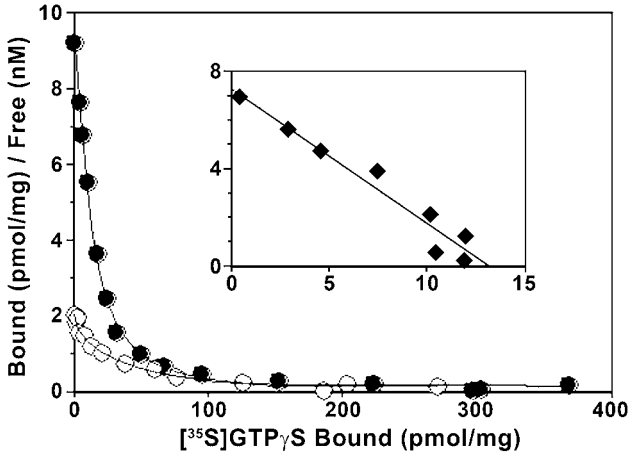

Figure 1. Scatchard plot of cannabinoid activation of [^{35}S]GTPγS binding in rat cerebellar membranes, showing basal (open symbols) and activated (closed symbols) [^{35}S]GTPγS binding, as determined with 3 μM WIN 55212-2. Inset shows net agonist-stimulated [^{35}S]GTPγS binding, with a high affinity of 2.7 nM compared with an affinity of 540 nM in the basal state. Adapted from Sim et al.[24]

nanomolar (nM). When WIN 55212-2 is added, there is an appearance of a substantial high affinity site for [^{35}S]GTPγS, with a K_D of 2.7 nM. Thus, WIN 55212-2 produces a 200-fold increase in the affinity of G_α for GTP.[24] The energy associated with this change in affinity is largely responsible for activation of receptor-mediated signal transduction.

In brain membranes, cannabinoid-stimulated [^{35}S]GTPγS binding is especially high because of the relatively large number of cannabinoid receptors in brain.[25] However, the number of receptors present does not always directly translate into a larger response because of the phenomenon of catalytic amplification between receptors and G-proteins. Several studies have shown that one receptor can couple to many different G-proteins to amplify agonist response. For example, in brain, cannabinoid receptor coupling to G-proteins is relatively inefficient[26,27]: in striatum, each cannabinoid receptor activates only 3 G-proteins compared with 20 G-proteins for each μ and δ opioid receptor (Table 1). It is possible that this relatively low amplification is related to the high number of CB_1 receptors; since these receptors exist in such high number in the brain, a high amplification between the receptor and transducer may not be necessary. A detailed brain regional analysis of cannabinoid amplification[27] showed that the amplification between CB_1 receptors and G-proteins varied widely between regions, with the smallest amplification factor of 2 in regions such as frontal cortex, cerebellum, and hippocampus, and the largest amplification factor of 7 in hypothalamus (Figure 2). These results suggest that different behavioral effects of cannabinoids that are mediated in different brain regions may be less related to the number of receptors present, but rather to the level of coupling between receptors and signal-transduction systems.

Table 1. Catalytic Amplification Factors for Opioid- and Cannabinoid-Stimulated G-Protein Activation in Rat Striatal Membranes*

Receptor	Receptor B_{max} pmol/mg	G-Protein B_{max} pmol/mg	Amplification Factor
μ Opioid	0.30	5.15	17
δ Opioid	0.29	6.27	22
Cannabinoid	3.56	10.05	3

*Amplification factors were calculated as ratios of B_{max} values of receptor binding to agonist-stimulated [^{35}S]GTPγS binding assays in rat striatal membranes. Adapted from.[26]

Agonist-stimulated [^{35}S]GTPγS binding can also be used to determine differences in agonist efficacy at the level of G-protein activation.[26,28] Using this technique in brain membranes,[24,29,30] WIN 55212-2 and levonantradol are full agonists, while anandamide produces partial efficacy, and Δ^9-tetrahydrocannabinol (Δ^9-THC) is a weak partial agonist (Figure 3). The discovery that anandamide is a partial agonist was surprising; traditionally, endogenous agonists in any neurotransmitter-receptor system are considered, by definition, full agonists. It is important to note that the lower efficacy of anandamide is not related to the metabolic instability of anandamide, since its metabolically stable analog methanandamide also produces the same partial efficacy as anandamide itself.

The efficacies of both exogenous and endogenous cannabinoids in activating G-proteins is related not only to the drugs ability to convert G_α into a high affinity state for GTP, but also to their ability to shift G_α into a low affinity state for GDP.[24] This principle is illustrated in Table 2, where the

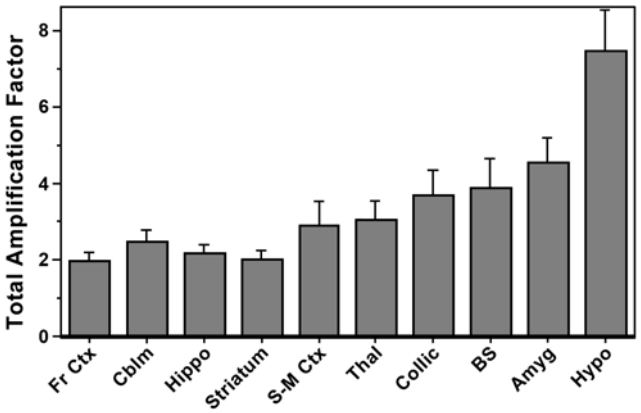

Figure 2. Brain regional variation in catalytic amplification of cannabinoid-activated G-proteins in rat brain. Amplification factors were calculated by comparing the ratios between B_{max} values of [^{3}H]SR141716A binding and WIN 55212-2-stimulated [^{35}S]GTPγS binding in membranes from various rat brain regions. Fr Ctx indicates frontal cortex; Cblm, cerebellum; Hippo, hippocampus; S-M cortex, sensomotor cortex; Thal, thalamus; Collic, superior colliculus; BS, brainstem; Amyg, amygdala; and Hypo, hypothalalmus. Adapted from Breivogel et al.[27]

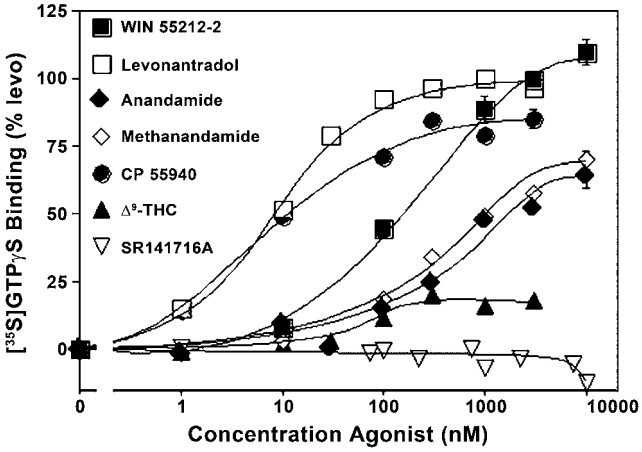

Figure 3. Cannabinoid agonist efficacies in activating G-proteins, as measured by agonist concentration-effect curves in stimulating [^{35}S]GTPγS binding in rat cerebellar membranes. Data are expressed as percentage of stimulation by the full agonist levonantradol. Adapted from Sim et al.[24]

efficacies of several cannabinoids (E_{max}) are related to their ability to shift G_α into low affinity states for GDP in cerebellar membranes. In the basal state, [^{35}S]GTPγS binding sites have 2 affinity states for GDP, 33 nM and 1147 nM. Additions of agonists have no effect on the high affinity GDP binding, but the efficacies of various cannabinoid agonists are directly related to their ability to shift the GDP low affinity state into even lower affinity. For example, the full agonist WIN 55212-2 shifts the GDP affinity from 1147 nM to 8210 nM, a decrease in affinity of 7.1-fold. A low efficacy partial agonist such as Δ^9-THC produces very little effect on the GDP affinity, decreasing affinity from 1147 nM to 1330 nM (1.2-fold shift). As predicted from its moderate efficacy, methanandamide produces a moderate change in the affinity for GDP, decreasing affinity from 1147 nM to 6570 nM (5.7-fold shift). These results confirm that anandamide is simply unable to produce a maximal activation of G-proteins, either by shifting G_α into a high affinity state for GTP, or shifting G_α into a low affinity state for GDP. This phenomenon is not just observed at the level of G-protein activation but also in the ability of anandamide to inhibit adenylyl cyclase[31] and affect ion channel function.[32,33] Why an endogenous ligand such as anandamide does not produce full efficacy at its receptor remains an unanswered question at this point. It is possible, however, that the relatively low efficacy of anandamide is counteracted by the large number of CB_1 receptors present in brain. Classical pharmacology predicts that partial agonists will exhibit full efficacy in the presence of a large receptor reserve, where less than full occupancy can produce a full agonist response.

There is a discrepancy in the actions of the CB_1 receptor antagonist SR141716A in [^{35}S]GTPγS experiments[24]; in rat cerebellar membranes, SR141716A is a neutral antagonist, with no effect on [^{35}S]GTPγS binding except at

Table 2. Relationship Between Cannabinoid Efficacy in Stimulating [^{35}S]GTPγS Binding, and Decreasing Affinities of GDP*

	E_{max} Values	GDP K_i Values (nM)	
Cannabinoid Agonist	**(% levo)**	**High Affinity**	**Low Affinity**
None (basal)	N/A	33 ± 5.1	1147 ± 141
Levonantradol	100 ± 5.9	34 ± 2.3	7730 ± 1030
WIN 55212-2	107 ± 2.3	39 ± 7.6	8210 ± 1190
CP 55940	81 ± 2.5	ND	ND
Anandamide	70 ± 5.8	ND	ND
Methanandamide	68 ± 2.1	33 ± 5.6	6570 ± 2540
Δ^9-THC	21 ± 0.7	20 ± 6.2	1330 ± 372

*Data compare the efficacies (E_{max} values) of various cannabinoid agonists determined from concentration-effect curves, with the affinity of GDP in displacing [^{35}S]GTPγS binding in the presence and absence of agonists. [^{35}S]GTPγS binding was performed in rat cerebellar membranes. Adapted from Sim et al.[24]

concentrations 10 000 times greater than its affinity at CB_1 receptors (Figure 3), while in CB_1 receptor-transfected cells, SR141716A is an inverse agonist, producing relatively potent inhibition of basal [^{35}S]GTPγS binding.[34] The signal of inverse agonists to reduce spontaneous activity of G-proteins is directly related to the number of receptors present, so that the detection of such activity is much more straightforward in transfected cells than in normal brain membranes. This fact demonstrates that findings of inhibition of basal [^{35}S]GTPγS binding in brain membranes by high concentrations of cannabinoid antagonists should be interpreted with caution.

Cannabinoid receptor activation of G-proteins influences multiple effector systems. Cannabinoid inhibition of adenylyl cyclase has been demonstrated in several cell types,[1,35,36] and in brain membranes.[31,37] In addition to inhibiting adenylyl cyclase, cannabinoids have been shown to stimulate cAMP accumulation.[38] As with other receptors coupled to $G_{i/o}$ proteins, activation of CB_1 receptors decreases Ca^{2+} conductance[32,39] and increases K^+ conductance.[40] Although beyond the scope of this review, retrograde signaling has been well documented as a mechanism of endogenous cannabinoid modulation of neuronal cell firing.[41]

Chronic Effects of Cannabinoids in Activating G-proteins

Chronic administration of cannabinoids to animals results in tolerance to many of the acute effects of Δ^9-THC, including memory disruption,[42] decreased locomotion,[43] and analgesia.[44] Several groups have attempted to correlate behavioral tolerance with biochemical alterations, and several studies have shown that brain cannabinoid receptor levels usually decrease after prolonged exposure to agonists,[45,46] although some studies have reported increases[47] or no changes[43] in receptor binding in brain. Appropriate controls have demonstrated that downregulation of cannabinoid receptors is homologous, and not simply due to neurotoxicity. Differences among studies may depend on the treatment agonist used, brain region examined, or treatment time. Despite these contradictory reports in vivo, there is general agreement that relatively short exposure of transfected cells in culture with cannabinoid agonists produces significant receptor internalization and trafficking.[48]

Another reason why reports of cannabinoid receptor downregulation have been contradictory is because receptor downregulation is only one consequence of receptor desensitization. For all G-protein-coupled receptors, the first step in desensitization is uncoupling of the receptor from G_α, thus reducing the agonist response. Therefore, the best place to look for chronic agonist-induced changes in receptor function is at the coupling between receptors and G-proteins. Chronic Δ^9-THC treatment produces significant desensitization of cannabinoid-activated G-proteins in several rat brain regions, as determined by cannabinoid-stimulated [^{35}S]GTPγS autoradiography.[30] These studies showed significant reduction in cannabinoid-stimulated [^{35}S]GTPγS binding in virtually every brain region (Figure 4), although the actual amount of desensitization varied across brain regions, with a maximum of 75% reduction in hippocampus. Moreover, the time course of the decrease in cannabinoid-stimulated [^{35}S]GTPγS binding varied across brain regions.[49] For example, the rate of desensitization was relatively fast in hippocampus, where significant reductions in cannabinoid-stimulated [^{35}S]GTPγS binding were observed after only 3 days of treatment with Δ^9-THC. A slower rate of desensitization was observed in cerebellum, where 7 days of treatment was required to see significant desensitization, while the slowest results were obtained in globus pallidus, where 14 days of chronic Δ^9-THC treatment were required for significant cannabinoid/G-protein desensitization. Such brain regional variation is consistent with the fact that tolerance to chronic drug exposure often develops at different rates for different behavioral effects. Other studies have confirmed the reduction in cannabinoid-activated G-proteins in brain following chronic

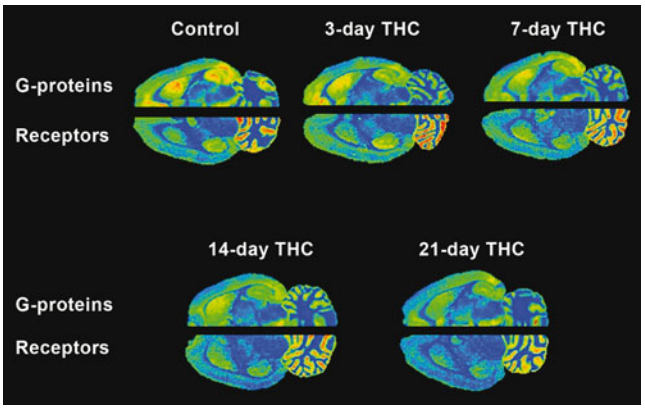

Figure 4. Effect of chronic treatment of rats with Δ^9-THC on WIN 55212-2-stimulated [^{35}S]GTPγS binding (top) and [^{3}H]SR141716A binding, determined by autoradiography of brain sections. Rats were treated with 10 mg/kg Δ^9-THC for 3 to 21 days. Note the time-dependent reductions in [^{35}S]GTPγS binding in hippocampus, caudate, globus pallidus, and cerebellum. Adapted from Breivogel et al.[49]

treatment with several cannabinoid agonists, including Δ^9-THC,[50-52] WIN 5521-2,[51] and CP-55940.[53] Of interest, in a study comparing the chronic effects of Δ^9-THC and WIN 55212-2, both agonists produced significant reduction in cannabinoid-stimulated [^{35}S]GTPγS binding throughout brain, with chronic Δ^9-THC even producing somewhat more desensitization than chronic WIN 55212-2 in some brain regions,[51] despite the fact that WIN 55212-2 has more efficacy than Δ^9-THC in activating G-proteins.

The relationship between in vivo tolerance and the uncoupling of cannabinoid receptors to G-proteins observed after chronic administration of cannabinoid agonists is not yet clear.[54] The phenomenon of tolerance is complex and involves not only specific cannabinoid receptor mechanisms, but also interactions between cannabinoid systems and other neurotransmitters in brain circuitry. Nevertheless, the loss of receptor/G-protein coupling represents a fundamental alteration of cannabinoid-induced signal transduction and is consistent with the loss of agonist response that characterizes cannabinoid tolerance.

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