0026-895X/00/051045-06$3.00/0
MOLECULAR PHARMACOLOGY

MOL 57:1045–1050, 2000 /13156/822093

Endocannabinoid 2-Arachidonyl Glycerol Is a Full Agonist through Human Type 2 Cannabinoid Receptor: Antagonism by Anandamide

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Received December 7, 1999; accepted February 3, 2000

This paper is available online at http://www.molpharm.org

ABSTRACT

The endocannabinoids anandamide and 2-arachidonyl glycerol (2-AG) bind to G protein-coupled central and peripheral cannabinoid receptors CB1 and CB2, respectively. Due to the relatively high expression of the CB2 isotype on peripheral immune cells, it has been hypothesized that this receptor mediates the immunosuppressive effects of cannabinoids. Unfortunately, there was a dearth of pharmacological studies with the endocannabinoids and human CB2 (hCB2). These studies compare and contrast the potency and efficacy of anandamide, 2-AG, and the synthetic cannabinoid HU210 at hCB2. Using [^{35}S]guanosine-5′-*O*-(3-thio)triphosphate (GTPγS) and radioligand bindings in insect Sf9-hCB2 membranes, we showed that both endocannabinoids bound hCB2 with similar affinity and that the cannabinoids acted as full agonists in stimulating [^{35}S]GTPγS exchange, although 2-AG was 3-fold more potent than anandamide (EC_{50} = 38.9 ± 3.1 and 121 ± 29 nM, respectively). In a mammalian expression system (Chinese hamster ovary-hCB2 cells), HU210 and 2-AG maximally inhibited forskolin-stimulated cAMP synthesis (IC_{50} = 1.61 ± 0.42 nM and 1.30 ± 0.37 μM, respectively) although anandamide was ineffective. In Chinese hamster ovary-hCB2 membranes, HU210 and 2-AG were also full agonists in stimulating [^{35}S]GTPγS binding (EC_{50} = 1.96 ± 0.35 and 122 ± 17 nM, respectively), but anandamide was a weak partial agonist (EC_{50} = 261 ± 91 nM; 34 ± 4% of maximum). Due to its low intrinsic activity, coincubation with anandamide effectively attenuated the functional activity of 2-AG at hCB2. Collectively, the data showed that both endocannabinoids bound hCB2 with similar affinity, but only 2-AG functioned as a full agonist. Moreover, the agonistic activity of 2-AG was attenuated by anandamide.

The arachidonic acid derivatives anandamide and 2-arachidonyl glycerol (2-AG) are endogenous ligands for the central and peripheral cannabinoid receptors CB1 and CB2, respectively (Devane et al., 1992; Mechoulam et al., 1995; Sugiura et al., 1995; for review, see Pertwee, 1997). Agonist activation of CB1 or CB2 inhibits adenylyl cyclase (Bayewitch et al., 1995; Slipetz et al., 1995) and stimulates mitogen-activated protein kinase (Bouaboula et al., 1995, 1996). Agonist binding to CB1 (but not CB2) inhibits voltage-activated calcium channels (Mackie and Hille, 1992) and can promote receptor interaction with both G_i and G_s in brain tissue (Glass and Felder, 1997; Felder et al., 1998). Differences in signal transduction coupling are not surprising because CB1 and CB2 share only 44% sequence similarity (Munro et al., 1993) and have distinct expression patterns. Originally cloned from rat brain (Matsuda et al., 1990; Gerard et al., 1991), CB1 is expressed primarily in the central nervous system and mediates many, if not all of the psychotropic and analgesic effects classically associated with cannabinoid agonists. CB2 was cloned from rat spleen and promyelocytic human leukemia 60 cells (Munro et al., 1993) and is expressed almost exclusively on peripheral immune cells (Galiegue et al., 1995; Schatz et al., 1997).

In addition to the aforementioned psychotropic and analgesic effects, recreational use of cannabinoids is associated with suppression of immune function (Kaminski, 1996, 1998; Klein et al., 1998). There have been a number of studies suggesting that endocannabinoids are also immunosuppressive (Cabral et al., 1995; Lee et al., 1995; Di Marzo et al., 1999). Due to its expression in immune cells, the immunomodulatory activities of cannabinoids have been largely attributed to activation of CB2. In spite of this, there is a dearth of pharmacological studies examining the intrinsic efficacies of endocannabinoids at human CB2. To this end, we initiated comparative characterization of 2-AG, anandamide, and the "classical" dibenzopyran cannabinoid HU210 with human CB2 (hCB2). We showed that although both endocannabinoids bound hCB2 with similar affinity, only 2-AG functioned as a full agonist in stimulating [^{35}S]guanosine-5′-*O*-(3-thio)triphosphate (GTPγS) exchange

ABBREVIATIONS: 2-AG, 2-arachidonyl glycerol; CB1, central cannabinoid receptor; CB2, peripheral cannabinoid receptor; hCB2, human type 2 cannabinoid receptor; GTPγS, guanosine-5′-*O*-(3-thio)triphosphate; CHO, Chinese hamster ovary; PMSF, phenylmethylsulfonyl fluoride; LPA, lysophosphatidic acid.

and inhibiting cAMP. Moreover, as a weak partial agonist at hCB2, anandamide attenuated 2-AG activation of hCB2.

Experimental Procedures

Cells and Cell Culture. The clonal Chinese hamster ovary (CHO)-hCB2 cell line was generated by transfection of CHO-K1 cells with hCB2 cDNA modified by placement of the hemagglutinin epitope on the N terminus cloned into the pCEP4 vector (Invitrogen, San Diego, CA). Stable clones were obtained by limiting dilution, screened with cannabinoid inhibition of cAMP, and confirmed by fluorescence-activated cell sorting analysis of cell surface hemagglutinin expression. Monolayer cultures were grown in T-175 flasks at 37°C in a humidified atmosphere (5% CO_2) in Dulbecco's modified Eagle's F-12 medium containing L-glutamine and supplemented with 1% nonessential amino acids, 1% penicillin/streptomycin, 10% fetal bovine serum (Gemini Biological Products, Calabasas, CA), and 0.2 mg/ml hygromycin B, pH 7.4. Experimental cultures were used 1 to 5 days after seeding. Cell culture medium was purchased from Life Technologies (Grand Island, NY).

CHO-hCB2 Membrane Preparation. CHO-hCB2 membranes were prepared as previously described (Hipkin et al., 1997). Briefly, cells at 75% confluence were harvested with cell dissociation buffer according to the manufacturer's instructions (Life Technologies). Cells were collected by centrifugation and used immediately or stored at −80°C. Cell pellets were resuspended and incubated on ice for 30 min in homogenization buffer (10 mM Tris-HCl, 5 mM EDTA, and 3 mM EGTA, pH 7.6) supplemented with 1 mM phenylmethylsulfonyl fluoride (PMSF) as a protease and amidase inhibitor (Pertwee et al., 1995; Compton and Martin, 1997). Cells were then homogenized with 20 strokes at 900 rpm with a Dounce homogenizer with stirrer type RZR1 polytron homogenizer (Caframo, Wiarton, Ontario, Canada). Intact cells and nuclei were removed by low-speed centrifugation (500*g* for 5 min at 4°C). Membranes in the supernatant were pelleted by centrifugation at 100,000*g* for 30 min at 4°C and then resuspended in gly-gly buffer (20 mM glycylglycine, 1 mM $MgCl_2$, and 250 mM sucrose, pH 7.2) and stored at −80°C. Protein determinations were performed with the Bradford method (Bradford, 1976).

[^{35}S]GTPγS and [^{3}H]CP55,940 Membrane Binding. Cell membranes (1–7 μg/point, in triplicate) were incubated in the presence or absence of various compounds for 30 min at 30°C in GTPγS binding buffer [20 mM HEPES, 100 mM NaCl, 5 mM $MgCl_2$, and 0.2% (w/v) BSA (Factor V, lipid free), pH 7.4] supplemented with 1 to 5 μM GDP. The reaction was carried out in 96-well microplates in a final volume of 100 μl. In [^{35}S]GTPγS binding experiments, the incubation included 0.3 nM [^{35}S]GTPγS (specific activity = 1250 Ci/mmol; NEN, Boston, MA). In radioligand competition assays, nonisotopic GTPγS was used and the reaction contained 1 to 2 nM [^{3}H]CP55,940 (specific activity = 180 Ci/mmol; NEN). The reaction was terminated by rapid filtration of the membranes through the microfiltration plates coated with 0.5% polyethylenimine (UniFilter GF/C filter plate; Packard, Meriden, CT) with a Tomtek 96-well cell harvester (Hamden, CT). In [^{35}S]GTPγS-binding experiments, the filters were washed 10 times at room temperature with 20 mM HEPES and 10 mM sodium pyrophosphate. For competition assays, the membranes were washed 10 times with ice-cold buffer composed of 50 mM Tris, 3 mM $MgCl_2$, 1 mM EDTA, and 0.1% (w/v) BSA, pH 7. Membrane-bound [^{35}S]GTPγS or [^{3}H]CP55,940 radioactivity was measured by liquid scintillation with a TopCount NXT microplate scintillation and luminescence counter (Packard). Nonlinear regression analysis of the data was performed with Prism 2.0b (GraphPad, San Diego, CA).

Intact Cell Radioligand Binding. Cells, seeded in 48-well plates, were chilled on ice and washed twice with cold binding buffer [F-12 nutrient mixture medium containing 10 mM HEPES and 0.2% (w/v) BSA, pH 7.4]. Cells were incubated overnight at 4°C in 200 μl of binding buffer containing various concentrations of [^{3}H]CP55,940 in the presence or absence of the indicated concentrations of cannabinoids. After repeated washes with cold binding buffer, the cells were lysed with 0.1 N NaOH and the solubolized radioligand measured by liquid scintillation. Specific binding in saturation analysis was calculated as the difference between the amount of radioligand bound in the absence (total binding) and presence of 1 μM HU210 (nonspecific binding).

cAMP Accumulation Assay. Cells, seeded in 96-well plates, were chilled on ice and washed twice with cold F-12 nutrient mixture medium containing 10 mM HEPES and 0.2% (w/v) BSA, pH 7.4. Cells were then incubated for 15 min at 37°C in the above-mentioned medium supplemented with 200 μM 3-isobutyl-1-methylxanthine (cAMP assay media), 5 μM forskolin, and the indicated concentrations of cannabinoids. The medium was removed and the cells lysed with 0.1 N HCl and rapid freezing. Intracellular cAMP in thawed lysates was measured by cAMP enzyme immunoassay (Biomol Re-

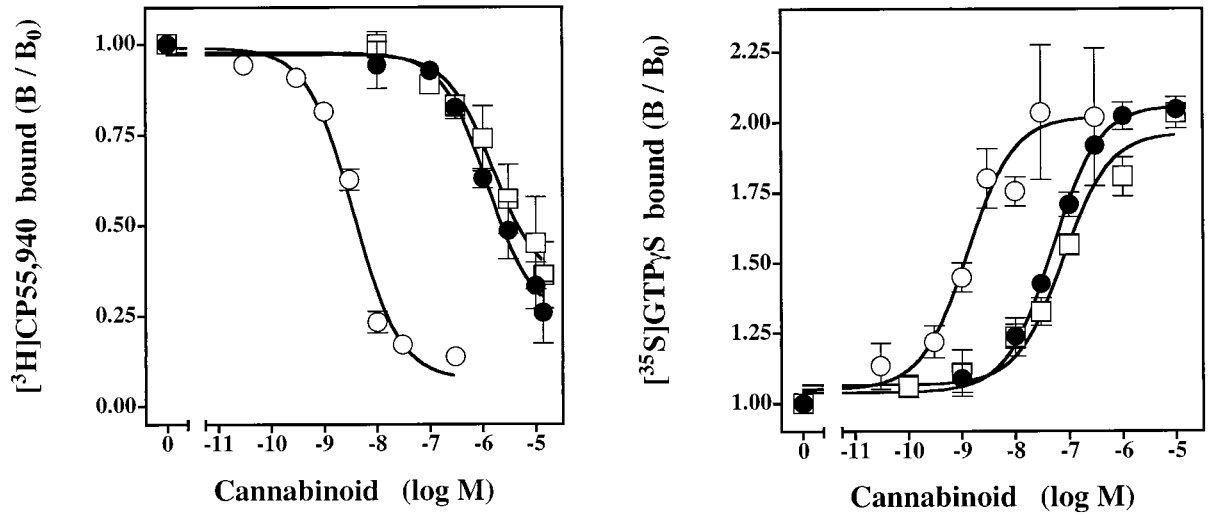

Fig. 1. Effect of cannabinoids on [^{35}S]GTPγS exchange and [^{3}H]CP55,940 binding in Sf9-hCB2 membranes. Membranes expressing hCB2 (4 μg/well) were incubated for 30 min at 30°C in GTPγS binding buffer (as described in *Experimental Procedures*) containing 1 μM GDP, the indicated concentrations of HU210 (○), 2-AG (●), or anandamide (□) and either 0.3 nM [^{35}S]GTPγS (right) or 0.3 nM GTPγS and 2 nM [^{3}H]CP55,940 (left). After filtration, membrane-associated radioactivity was measured by liquid scintillation. Data represent the mean total binding ± S.E. of triplicate determinations from three or four independent experiments.

search Laboratories, Plymouth Meeting, PA) according to manufacturer's instructions. The results are expressed as a fraction of forskolin-stimulated cAMP accumulation measured in the absence of cannabinoids.

Data Analysis. Data are reported as mean ± S.E. of at least three independent experiments, each of which was performed in triplicate. Nonlinear regression analysis of saturation data and of concentration-response data was performed with Prism 2.0b software (GraphPad) to calculate K_d, B_{max}, IC_{50}, and EC_{50}. IC_{50} values were converted to apparent K_i values by the method of Cheng and Prusoff (1973) with the K_d values for [^{3}H]CP55,940 determined from saturation experiments.

Materials. Sf9 membranes exogenously expressing $G\alpha_{i3}$, $\beta_1\gamma_2$, and hCB2 (7–14 pmol/mg) or hCB1 (0.7 pmol/mg) were purchased from NEN. HU210 was purchased from Biomol. 2-AG, anandamide, and *R*-(+)-methanandamide were obtained from Research Biochemicals/Sigma (Natick, MA). All other reagents were of the best grade available and purchased from common suppliers.

Results

Effect of Cannabinoids on [^{35}S]GTPγS Exchange and [^{3}H]CP55,940 Binding in Sf9-hCB2 Membranes. A [^{35}S]GTPγS exchange assay was instituted with membranes from Sf9 insect cells transfected to overexpress hCB2 and mammalian G proteins $G\alpha_{i3}$ and $\beta_1\gamma_2$. To estimate receptor coverage in these experiments, we performed radioligand-binding studies with assay conditions identical with those used for [^{35}S]GTPγS binding (as described in *Experimental Procedures*). Under these conditions, 2 nM [^{3}H]CP55,940 bound to equilibrium binding by 30 min ($t_{1/2}$ = 2.8 min; data not shown). Saturation-binding analysis revealed that [^{3}H]CP55,940 bound with expected affinity (K_d = 2.4 ± 0.05 nM; n = 2; data not shown). We then measured ligand affinities with radioligand competition assays (Fig. 1, left) and found that HU210 bound with considerably higher affinity (K_i = 2.3 ± 0.3 nM; n = 4) than did 2-AG and anandamide (K_i = 949 ± 270 and 795 ± 46 nM, respectively; n = 3). In parallel experiments, we measured [^{35}S]GTPγS exchange (as described in *Experimental Procedures*). HU210, 2-AG, and anandamide were all full agonists (Fig. 1, right) although neither endocannabinoid was as potent as HU210 (EC_{50} = 1.1 ± 0.2, 38.9 ± 3.1, and 121 ± 29 nM, respectively; n = 3–5).

CB2 Receptor Expression and Affinity in CHO-hCB2 Cells. We extended our studies with a mammalian expression system. Saturation-binding analysis with CHO-hCB2 cells at 4°C (Fig. 2, left) revealed that the CHO-hCB2 cells bound [^{3}H]CP55,940 with the expected high affinity (K_d = 4.6 ± 0.41 nM; Pertwee, 1997). Saturation analysis in membranes showed lower hCB2 expression in the CHO-hCB2 cells (7.2 ± 0.6 pmol/mg; n = 3) than in the Sf9 expression system (10–14 pmol/mg). Competition with [^{3}H]CP55,940 in intact CHO-hCB2 cells (Fig. 2, right) showed that HU210, 2-AG and anandamide bound with the following affinities: 4.9 ± 0.6 nM, 650 ± 115 nM, and 3.5 ± 0.3 μM, respectively.

Effect of Cannabinoids on Forskolin-Stimulated cAMP Accumulation in CHO-hCB2 Cells. The lower receptor expression and more physiologically relevant G protein complement suggested that a CHO-hCB2 cell model could be more amenable for distinguishing differences in the intrinsic efficacies of the endocannabinoids (Whaley et al., 1994; Krumins and Barber, 1997). To this end, CHO-hCB2 cells were incubated at 37°C for 15 min with 5 μM forskolin and the indicated concentrations of HU210, 2-AG, anandamide, or the metababolically stable anandamide congener *R*-(+)-methanandamide. As can be seen in Fig. 3, both HU210 and 2-AG maximally inhibited forskolin-stimulated cAMP synthesis (IC_{50} = 1.6 ± 0.4 nM, n = 9 and 1.3 ± 0.4 μM, n = 4, respectively). However, anandamide inhibited cAMP only slightly and only at very high concentrations (IC_{50} > 30 μM). *R*-(+)-Methanandamide also inhibited fors-

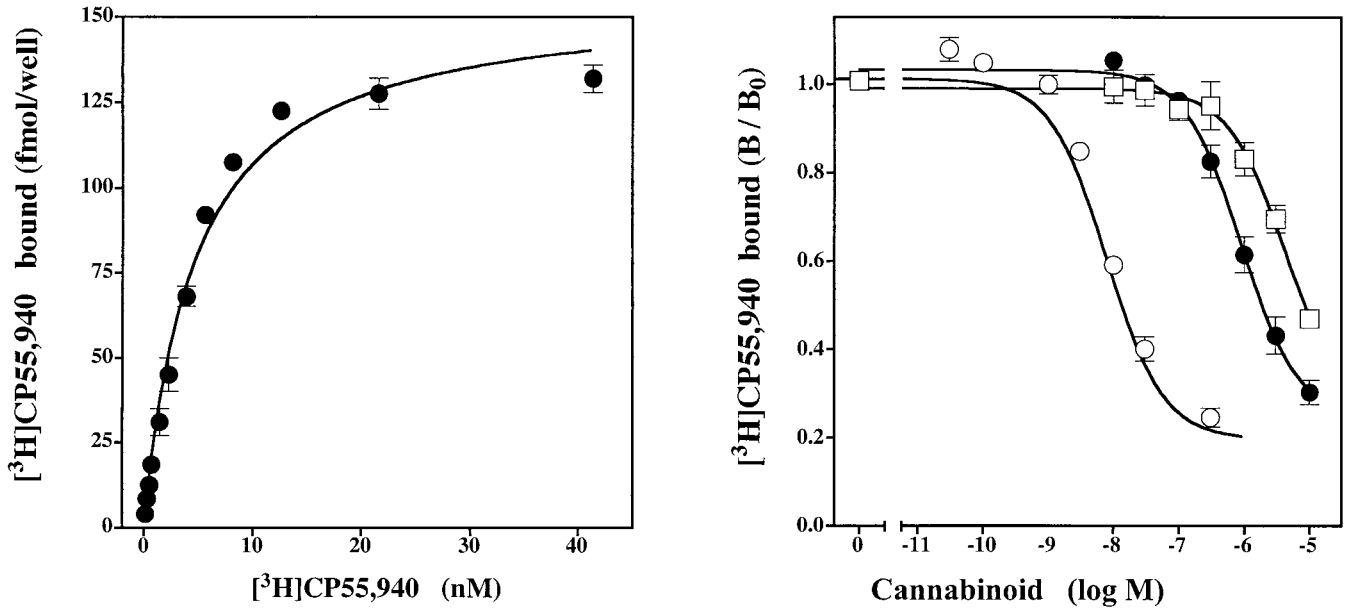

Fig. 2. Saturation and competition binding in intact CHO-hCB2 cells. Cells in 48-well plates were incubated overnight at 4°C with various concentrations of [^{3}H]CP55,940 in the presence or absence of 1 μM HU210 (left) or for competition bindings (right), with 2 nM [^{3}H]CP55,940 in the presence or absence of the indicated concentrations of HU210 (○), 2-AG (●), or anandamide (□). Cells were then washed repeatedly and lysed, and the bound radioligand was measured by liquid scintillation. Data represent the mean ± range of triplicate determinations from two or three independent experiments. Ligand affinities from competition bindings were calculated from binding IC_{50} with the Cheng-Prusoff equation.

kolin-stimulated cAMP somewhat, but again only at high concentrations (IC_{50} > 10 μM). This finding and the observation that pre- or co-incubation with 0.2 to 1 mM PMSF to inhibit endogenous amidases (Hillard et al., 1995; Pertwee et al., 1995) failed to increase the potency or the efficacy of either anandamide or 2-AG to inhibit cAMP suggests that endocannabinoid degradation was minimal. From these data, we conclude that 2-AG but not anandamide effectively inhibits cAMP signaling through hCB2.

Effect of Cannabinoids on [^{35}S]GTPγS Exchange and [^{3}H]CP55,940 Binding CHO-hCB2 Membranes. To further examine 2-AG and anandamide agonism, we initiated [^{35}S]GTPγS exchange and [^{3}H]CP55,940-binding studies. [^{3}H]CP55,940-binding equilibrium was attained by 30 min with a K_d = 0.97 ± 0.23 nM (n = 3; data not shown). Competition assays (Fig. 4, right) generated K_i values for HU210, 2-AG, and anandamide of 0.83 ± 0.17, 474 ± 92, and 348 ± 31 nM, respectively (n = 4–6). These affinities are all 2-fold higher than those measured in the Sf9-hCB2 membranes.

CHO-hCB2 membranes were incubated in the presence or absence of cannabinoids with 0.3 nM [^{35}S]GTPγS. As was the case in the insect expression system, HU210 was the most potent agonist (Fig. 4, left; EC_{50} = 1.96 ± 0.35 nM; n = 6). Again, 2-AG was also a full agonist in stimulating [^{35}S]GTPγS binding with an EC_{50} = 122 ± 17 nM (n = 5). However, anandamide was a weak partial agonist in CHO-hCB2 membranes (34 ± 4% of maximum) with an EC_{50} = 261 ± 91 nM (n = 4).

Because weak agonists can antagonize receptor activation by a stronger agonist, we examined the effect of anandamide on 2-AG-stimulated [^{35}S]GTPγS binding. Membranes were incubated in the presence or absence of 0.1, 1.0, or 10 μM anandamide and 100 nM 2-AG. Coincubation with anandamide decreased the efficacy of 2-AG to stimulate 2-AG-stimulated [^{35}S]GTPγS binding in a dose-dependent manner (Fig. 5, left). Two approaches were taken to ensure that this effect was specific to anandamide and hCB2. First, parental CHO-K1 membranes were coincubated with 10 μM anandamide and lysophosphatidic acid (LPA) to assess the effect of the endocannabinoid on [^{35}S]GTPγS binding in the absence of measurable cannabinoid receptor expression. As can be seen in Fig. 5 (right), anandamide had no effect on LPA-stimulated [^{35}S]GTPγS binding (n = 2). Similarly, 10 μM anandamide did not effect the stimulation of [^{35}S]GTPγS binding in BaF membranes expressing CXCR3 by the chemokine agonist human interferon γ-inducible protein-10 (data not shown). From these data, we conclude that the effect of anandamide on 2-AG-stimulated [^{35}S]GTPγS binding is dependent on the expression of hCB2. We next examined the effect of 10 μM arachidonic acid, an anandamide metabolite, on 2-AG-stimulated [^{35}S]GTPγS binding in CHO-hCB2 membranes and found that coincubation with arachidonic acid had no effect (data not shown). From these data, we conclude that anandamide rather than its metabolite arachidonic acid antagonizes hCB2 activation by 2-AG. Collectively, we can conclude from these data that, as a weak partial agonist, anandamide can function as an endogenous antagonist at the peripheral cannabinoid receptor.

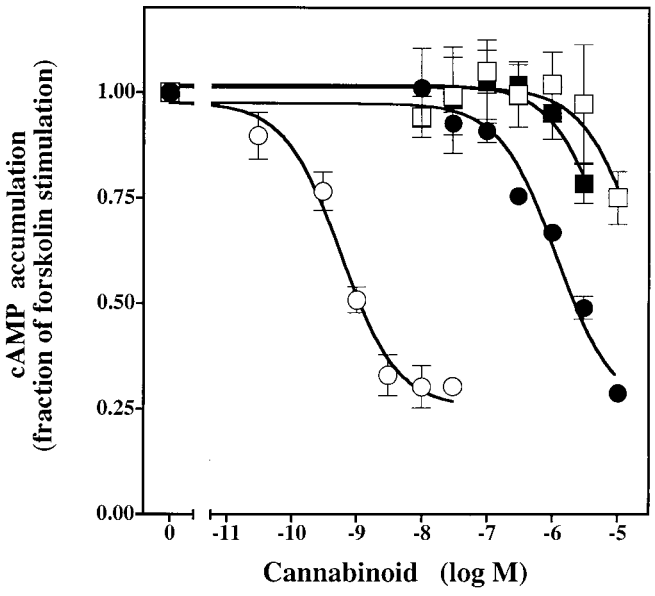

Fig. 3. Effect of cannabinoids on cAMP accumulation in intact CHO-hCB2 cells. Cells in 96-well plates were incubated for 15 min at 37°C in cAMP assay buffer (as described in *Experimental Procedures*) containing 5 μM forskolin and the indicated concentrations of HU210 (○), 2-AG (●), anandamide (□), or *R*-(+)-methanandamide (■). After incubation, intracellular cAMP was measured by enzyme immunoassay. Data represent the mean fraction of forskolin-stimulated cAMP ± range of triplicate determinations from two to five independent experiments (HU210 and 2-AG) or from a representative experiment (anandamide).

Discussion

These studies demonstrated that 2-AG is a full agonist through hCB2 in stimulating GTPγS exchange in membranes and inhibiting cAMP in intact cells. Furthermore, we showed that anandamide is a weak partial agonist in these systems and as such, could antagonize hCB2 activation by 2-AG. Collectively, activation of the peripheral cannabinoid receptor, be it by 2-AG or any effective agonist, could be tempered by the local concentrations of anandamide.

In some tissues, the potency of anandamide was increased in the presence of PMSF through irreversible inhibition of endogenous amidases that hydrolyze anandamide to arachidonic acid and ethanolamine (Hillard et al., 1995; Pertwee et al., 1995). It was unlikely that the effectiveness of anandamide in CHO-hCB2 was similarly influenced by amidase degradation because neither pre- nor co-incubation of intact cells with PMSF had any effect on the potency or efficacy of anandamide or 2-AG to inhibit cAMP accumulation (data not shown). Furthermore, the metabolically stable anandamide analog *R*-(+)-methanandamide was a poor agonist in inhibiting cAMP in CHO-hCB2 cells and antagonized 2-AG-stimulated GTPγS exchange in CHO-hCB2 membranes (data not shown). Moreover, 2-AG also would be susceptible to amidase degradation as has been shown in other tissues (Goparaju et al., 1998); however, 2-AG was fully effective in inhibiting forskolin-stimulated cAMP accumulation. Our anandamide preparation was bioactive because it acted as a full agonist in stimulating GTPγS exchange in Sf9-hCB1 membranes and was 20-fold more potent than 2-AG (EC_{50} anandamide = 78 ± 22 nM; 2-AG = 1.6 ± 0.55 μM; n = 3–4; data not shown). Interestingly, anandamide was effective in stimulating GTPγS exchange in Sf9-hCB2 insect cell membranes. The increased intrinsic efficacy of anandamide in this system most likely reflected the higher expression of hCB2 and G

proteins relative to that in the CHO-hCB2 cells. Barber and coworkers (Whaley et al., 1994; Krumins and Barber, 1997) clearly demonstrated that partial agonists can appear to be full agonists as receptor or G-protein expression was increased.

It is tempting to speculate that as a full agonist at hCB2, 2-AG could be an endogenous modulator of human immune function as was previously postulated based on studies with murine splenocytes (Mechoulam et al., 1995). 2-AG has been reported to inhibit mixed lymphocyte response, T-cell proliferation, and lipopolysaccharide-induced B-cell proliferation in the mouse (Lee et al., 1995). More recently, it was shown that 2-AG suppressed interleukin-2 secretion in mouse splenocytes (Ouyang et al., 1998). However, because mouse spleen contains both CB1 and CB2 mRNA (data not shown; Kaminski et al., 1992), the inhibitory effects of 2-AG on cAMP accumulation and immune function in this model may represent activation of mouse CB1 and/or CB2. Moreover, mouse CB2 differs from hCB2 in 60 residues and has a truncated C-terminal tail (Pertwee, 1997). Due to the considerable difference in receptor structure between the human and mouse homolog (82% similar), 2-AG activity through rodent CB2 does not a priori define activity at the human receptor. Incubation of mouse macrophages with lipopolysaccharide stimulated a 2- and 7.8-fold increase in the levels of 2-AG and anandamide, respectively (Di Marzo et al., 1999). However, our data demonstrated that anandamide is a poor agonist at hCB2. Therefore, anandamide is not likely to act in

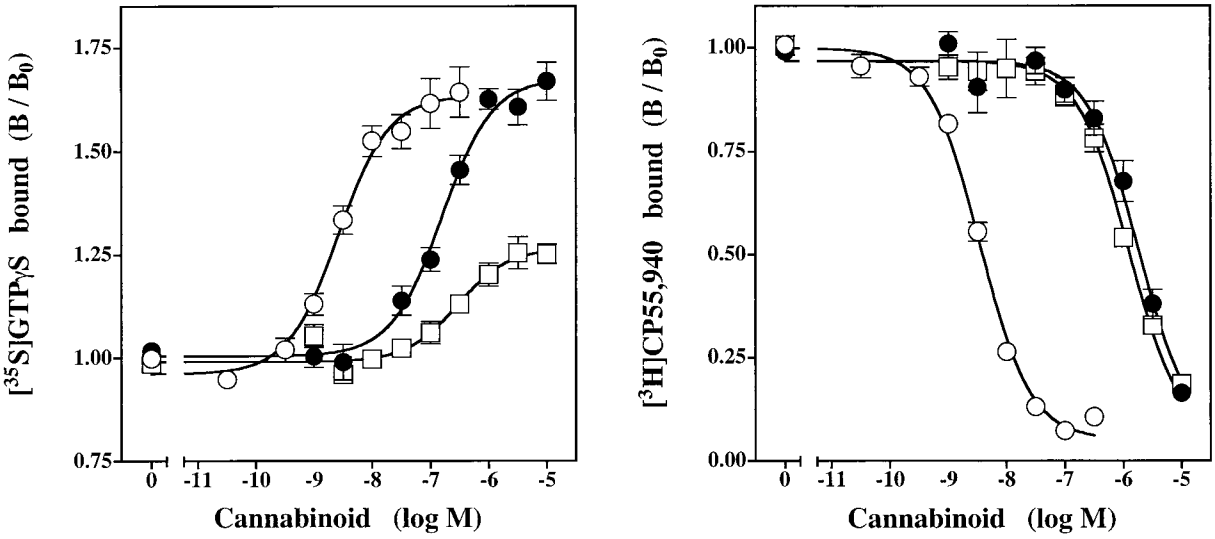

Fig. 4. Effect of cannabinoids on [^{35}S]GTPγS exchange and [^{3}H]CP55,940 binding in CHO-hCB2 membranes. Membranes (4 μg/well) were incubated for 30 min at 30°C in GTPγS binding buffer (as described in *Experimental Procedures*) containing 5 μM GDP, the indicated concentrations of HU210 (○), 2-AG (●), or anandamide (□), and either 0.3 nM [^{35}S]GTPγS (left) or 0.3 nM GTPγS and 2 nM [^{3}H]CP55,940 (right). After filtration, the membrane-associated radioactivity was measured by liquid scintillation. Data represent the mean ± S.E. of triplicate determinations from three independent experiments and are expressed as a fraction of basal binding.

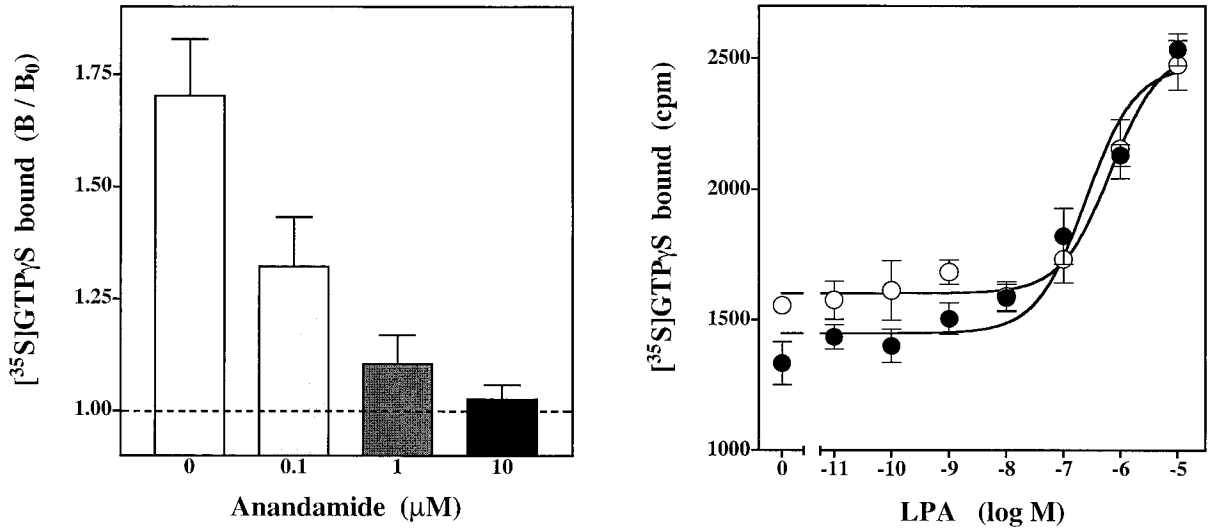

Fig. 5. Effect of anandamide on 2-AG- or LPA-stimulated [^{35}S]GTPγS exchange. CHO-hCB2 (left) or CHO-K1 (right) membranes (3 μg/well) were incubated for 30 min at 30°C in GTPγS binding buffer (as described in *Experimental Procedures*) containing 5 μM GDP, 0.3 nM [^{35}S]GTPγS with 100 nM 2-AG, and the indicated concentrations of anandamide (left) or LPA (right) in the absence (●) or presence (○) of 10 μM anandamide. After filtration, the membrane-associated radioactivity was measured by liquid scintillation. Data represent the mean total binding ± range of triplicate determinations from two to four independent experiments.

vivo as an immunosuppressant through CB2. Indeed, Lee et al. (1995) found that anandamide had no effect on immune function in B6C3F1 mouse splenocytes. Recently, anandamide was reported to be a synergistic growth factor in murine hematopoietic cells expressing CB2, although this activity was not duplicated by other cannabinoids (Valk et al., 1997; Derocq et al., 1998). A subsequent study by Derocq et al. (1998) showed that the enhancement of hematopoietic cell growth by anandamide was not blocked by pertussis toxin pretreatment nor by the cannabinoid receptor antagonists SR141716A and SR144528. It was concluded therefore that the effect of anandamide on cell growth was not mediated through cannabinoid receptors. As a poor agonist at hCB2, anandamide attenuated the effectiveness of 2-AG to activate hCB2 (Fig. 5). In an analogous manner, the immunosuppressive effects in vivo of 2-AG or other cannabinoids could be attenuated by anandamide. Indeed, studies showed that anandamide attenuated the stimulation of serotonin secretion by other cannabinoids in CB2-expressing rat basophilic leukemia-2H3 cells (Facci et al., 1995). Therefore, if this hypothesis is correct, immunomodulation by endocannabinoids in the periphery would depend on the local concentration of both 2-AG and anandamide.

From these studies, we conclude that 2-AG is a full agonist at the human peripheral cannabinoid receptor. Moreover, we conclude that anandamide is much less effective at activating this receptor and can functionally antagonize the stimulatory effects of 2-AG at hCB2.

Acknowledgments

We thank Dr. R. Kyle Palmer and Kaitlin Hipkin for critical reading of this manuscript.

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