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Rapid Communication

SR141716A is an inverse agonist at the human cannabinoid CB_1 receptor

Robert S. Landsman [a,b,c,d,e], Thomas H. Burkey [a,b,c,d,e], Paul Consroe [a,b,c,d,e], William R. Roeske [a,b,c,d,e], Henry I. Yamamura [a,b,c,d,e,*]

[a] *Department of Pharmacology, University of Arizona Health Sciences Center, Tucson, AZ 85724, USA*
[b] *Department of Pharmacology and Toxicology, University of Arizona Health Sciences Center, Tucson, AZ 85724, USA*
[c] *Department of Biochemistry, University of Arizona Health Sciences Center, Tucson, AZ 85724, USA*
[d] *Department of Psychiatry, University of Arizona Health Sciences Center, Tucson, AZ 85724, USA*
[e] *Program in Neuroscience, University of Arizona Health Sciences Center, Tucson, AZ 85724, USA*

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Abstract

The effects of *R*(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-*de*]-1,4-benzoxazin-yl]-(1-napthalenyl)methanone mesylate (WIN 55,212-2) and *N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide (SR141716A) on guanosine-5′-*O*-(3-[^{35}S]thio)triphosphate ([^{35}S]GTPγS) binding to membranes isolated from human cannabinoid CB_1 receptor-transfected Chinese hamster ovary (CHO) cells were examined. WIN 55,212-2 stimulated [^{35}S]GTPγS binding 76.3% above basal levels whereas SR141716A produced a 22.3% decrease in basal [^{35}S]GTPγS binding. These findings demonstrate that WIN 55,212-2 is an agonist and SR141716A is an inverse agonist in this system. © 1997 Elsevier Science B.V.

Keywords: SR141716A; Inverse agonist; Cannabinoid receptor, human

The aminoalkylindole, *N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide (SR141716A), has been previously characterized as an antagonist at the cannabinoid CB_1 receptor (Rinaldi-Carmona et al., 1994). These investigators found that SR141716A displaced cannabinoid agonists in membranes prepared from rat brain synaptosomes or cannabinoid CB_1 receptor-transfected Chinese hamster ovary (CHO) cells; however, SR141716A poorly displaced cannabinoid drugs in membrane preparations from spleen or cannabinoid CB_2 receptor-transfected CHO cells. SR141716A blocked *R*(+)-[2,3-dihydro-5-methyl-3[(morpholinyl)methyl]pyrrolo[1,2,3-*de*]-1,4-benzoxazin-yl]-(1-napthalenyl)methanone mesylate (WIN 55,212-2)-mediated inhibition of forskolin-stimulated adenylyl cyclase activity in preparations from rat substantia nigra synaptosomes and antagonized several in vivo measures of cannabinoid activity in WIN 55,212-2-treated rodents. However, SR141716A had no effect on these functions alone suggesting that it is a neutral antagonist in vivo.

Intravenous injection of SR141716A in mice inhibited Δ^9-THC-induced hypoactivity, hypothermia and antinociception at doses ≤ 3 mg/kg. However, above this dose, SR141716A alone stimulated locomotor activity (Compton et al., 1996). Richardson et al. (1997) showed that intrathecal injection of SR141716A evoked a significant thermal hyperalgesia in mice. In contrast to the data of Rinaldi-Carmona et al. (1994), these data suggest the possibility that SR141716A blocks the activity of an endogenous ligand or acts as an inverse agonist. We thus examined the effect of SR141716A on [^{35}S]GTPγS binding in membranes prepared from CHO cells stably expressing the human cannabinoid CB_1 receptor at 2.6 pmol/mg membrane protein. The results show that SR141716A is an inverse agonist in this system.

The human cannabinoid CB_1 receptor cDNA (a gift from Dr. Marc Parmentier, Bruxelles, Belgium) was inserted into the pHβAPr-1-neo expression vector (a gift

* Corresponding author. Tel.: +1-520-6267381; fax: +1-520-6262204; e-mail: hiy@ccit.arizona.edu.

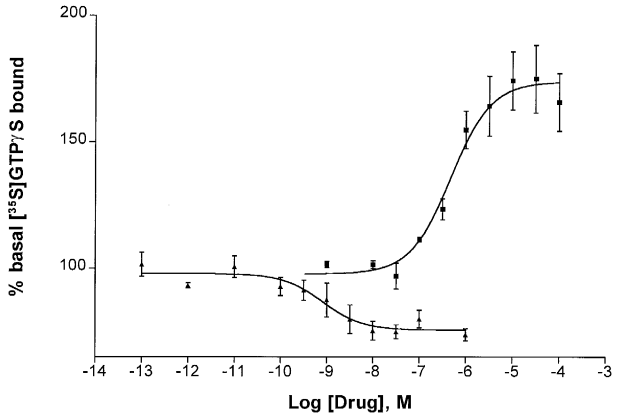

Fig. 1. Effect of WIN 55,212-2 ($n = 4$; closed squares) and SR141716A ($n = 4$; closed triangles) on 0.1 nM [^{35}S]GTPγS binding to membranes prepared from CHO cells stably expressing the human cannabinoid CB_1 receptor. Symbols represent the mean response and the vertical lines indicate the S.E.M. Drugs were initially dissolved in 50% ethanol to produce a 1 mM stock solution. Further dilutions were done using GTPγS buffer.

from Dr. L. Kedes, Stanford University) and stably expressed in CHO cells using the cationic lipid *N*-[1-(2,3-dioleoyloxy)propyl]-*N*,*N*,*N*-trimethylammonium methylsulfate (DOTAP, Boehringer Mannheim, Indianapolis, IN). Confluent monolayers of stably transfected CHO cells were washed with Ca^{2+} and Mg^{2+}-free phosphate-buffered saline (PBS), incubated for 5 min at 37°C in PBS containing EDTA (1.0 mM), scraped and pelleted ($1500 \times g$). Cells were resuspended in GTPγS buffer (25 mM Tris–HCl, 150 mM NaCl, 2.5 mM $MgCl_2$, 1 mM EDTA, 0.25% bovine serum albumin, 50 μM GDP, 30 μM bestatin, 10 μM captopril and 0.1 mM phenylmethylsulfonyl fluoride, pH = 7.4), homogenized with a Dounce homogenizer (10 strokes), pelleted ($40{,}000 \times g$) and resuspended in GTPγS buffer to prepare a crude membrane fraction.

Cannabinoid CB_1 receptor-mediated modulation of G protein activity was determined as [^{35}S]GTPγS binding (Traynor and Nahorski, 1995; Selley et al., 1996). Assay tubes contained final concentrations of 0.1 nM [^{35}S]GTPγS (1250 Ci/mmol, DuPont NEN, Boston, MA), 0.1% tissue (w/v), either WIN 55,212-2 (1.0–100,000 nM, Research Biochemicals, Natick, MA) or SR141716A (0.0001–1,000 nM, a gift from Gerard Le Fur, Sanofi Recherche, Montpellier) and were brought to a final volume of 1 ml using GTPγS buffer. Tubes were incubated at 30°C for 90 min and filtered using a 48 channel Brandel harvester onto GF/B filters presoaked in GTPγS buffer. Filters were washed four times (25 mM Tris–HCl, 120 mM NaCl), stored at 4°C overnight and the radioactivity was determined by scintillation spectrophotometry.

As seen in Fig. 1, WIN 55,212-2 stimulated [^{35}S]GTPγS binding to 176% of basal levels with an EC_{50} of 473 nM. In contrast, SR141716A inhibited basal [^{35}S]GTPγS binding by 22.3% with an EC_{50} value of 0.82 nM. The ability of SR141716A to reduce basal G protein activity suggests that both SR141716A-mediated increases in locomotor activity and hyperalgesia in mice may be due to inverse agonist activity of the drug as opposed to an antagonistic effect on endogenous cannabinoid-mediated homeostatic mechanisms.

In conclusion, SR141716A is an inverse agonist in membranes prepared from human cannabinoid CB_1 receptor-transfected CHO cells as assessed by the [^{35}S]GTPγS assay. In contrast, WIN 55,212-2 is an agonist in this system. Potential uses of inverse cannabinoid agonists can be seen in studies by Terranove et al. (1996) where SR141716A reduced memory deficits in aged rats and improved social recognition in adult rats. The development of other compounds with greater inverse agonist activity can be investigated using SR141716A as the prototype.

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