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# $\Delta^9$-Tetrahydrocannabinol is a partial agonist of cannabinoid receptors in mouse brain

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**Abstract**

We measured the ability of the cannabinoid agonists $\Delta^9$-tetrahydrocannabinol and *R*(+)-[2,3,-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-*de*]-1,4-benzoxazin-yl]-(1-naphthalenyl) methanone mesylate (WIN 55,212-2) to stimulate guanosine-5′-*O*-(3-[$^{35}$S]thio)triphosphate ([$^{35}$S]GTPγS) binding in mouse brain membranes. $\Delta^9$-Tetrahydrocannabinol stimulated [$^{35}$S]GTPγS binding by about 25% as compared to WIN 55,212-2. This is the first report demonstrating that $\Delta^9$-tetrahydrocannabinol acts as a partial agonist in stimulating [$^{35}$S]GTPγS binding in the mouse brain.

*Keywords:* Brain, mouse; $\Delta^9$-Tetrahydrocannabinol; G-protein

It is well documented that cannabinoid drugs provide relief of pain as well as display antiemetic properties (Dewey, 1986). These drugs are known to bind in the brain to a G protein coupled receptor referred to as the cannabinoid $CB_1$ receptor (Matsuda et al., 1990; Howlett, 1995). The cannabinoid $CB_1$ receptor has been cloned and when the cDNA is transfected into Chinese hamster ovary (CHO-K1) cells in vitro, cannabinoids inhibit forskolin-stimulated intracellular cAMP levels through pertussis toxin sensitive G proteins (Matsuda et al., 1990). Cannabinoid receptors have also been demonstrated to be coupled to N-type calcium and potassium channels by a pertussis toxin sensitive pathway (Howlett, 1995).

Cannabinoid drugs are now divided into 4 classes: (1) classical cannabinoids having structures similar to $\Delta^9$-tetrahydrocannabinol; (2) cannabinoids with bicyclic structures, such as CP 55,940; (3) aminoalkylindoles, such as *R*(+)-[2,3,-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-*de*]-1,4-benzoxazin-yl]-(1-naphthalenyl) methanone mesylate (WIN 55,212-2); and (4) arachidonic acid metabolites, such as anandamide. The pharmacological properties of over 300 cannabinoid compounds have been documented (Razdan, 1986); however, simple functional in vitro screening methods for drug activity have been lacking. As cannabinoid receptors in vivo are coupled to antinociception through pertussis toxin sensitive G proteins (Lichtman et al., 1996), in vitro assays of G protein activity should predict cannabinoid drug activities in whole animal studies. Recently an assay has been developed to measure cannabinoid receptor activation of G proteins by determination of the binding of the GTP analogue [$^{35}$S]GTPγS to brain membranes (Selley et al., 1996). Using similar methods we demonstrate that $\Delta^9$-tetrahydrocannabinol is a partial agonist of cannabinoid-stimulated G protein activation in membranes isolated from mouse brain.

Male (Institute for Cancer Research, ICR) mice, weighing 20–30 g (Harlan Sprague Dawley, Indianapolis, IN, USA) were killed by cervical dislocation. Whole brains were removed and homogenized in TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.4) buffer. After centrifugation the membrane pellet was resuspended in assay buffer (25 mM Tris-HCl, 150 mM NaCl, 2.5 mM $MgCl_2$, 1 mM EDTA, 50 μM GDP, 0.25% bovine serum albumin, 30 μM bestatin, 10 μM captopril and 0.1 mM phenylmethylsulfonyl fluoride, pH 7.4) and incubated (30 min, 30°C) to degrade endogenous ligands. Membranes were then cen-

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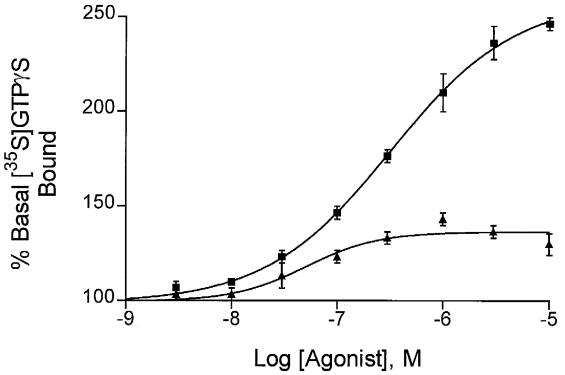


Fig. 1. Induction of [$^{35}$S]GTPγS binding to membranes isolated from whole mouse brain upon stimulation with increasing concentrations of $\Delta^9$-tetrahydrocannabinol (▲) or WIN 55,212-2 (■). $EC_{50}$ values were 59 and 347 nM for $\Delta^9$-tetrahydrocannabinol and WIN 55,212-2 in stimulating [$^{35}$S]GTPγS binding, respectively. Ethanol was present at 0.5% at the highest concentration of both agonists used in this study. Data are expressed as the mean ± S.E.M. ($n = 3$ for both agonists).

trifuged, resuspended in assay buffer and incubated with increasing concentrations of $\Delta^9$-tetrahydrocannabinol or WIN 55,212-2 (both from Research Biochemicals International, Natick, MA, USA) in the presence of 0.1 nM [$^{35}$S]GTPγS (specific activity 1000–1500 Ci/mmol, DuPont NEN, Boston, MA, USA). After incubation (90 min, 30°C), the reaction was terminated by rapid filtration under vacuum through Whatman GF/B glass fiber filters, followed by four washes with ice-cold 25 mM Tris/120 mM NaCl, pH 7.4. Bound radioactivity was measured by liquid scintillation spectrophotometry after an overnight extraction with EcoLite (ICN Biomedicals, Aurora, OH, USA) scintillation cocktail. Data were analyzed using Prism (GraphPad, San Diego, CA, USA).

Both $\Delta^9$-tetrahydrocannabinol and WIN 55,212-2 stimulated [$^{35}$S]GTPγS binding to membranes isolated from the mouse brain (Fig. 1). The $EC_{50}$ values for $\Delta^9$-tetrahydrocannabinol and WIN 55,212-2 were 59 and 347 nM, respectively. Maximal $\Delta^9$-tetrahydrocannabinol stimulation of [$^{35}$S]GTPγS binding was about 25% of that observed with WIN 55,212-2. This difference in maximal stimulation is not due to differential binding of $\Delta^9$-tetrahydrocannabinol and WIN 55,212-2 to cannabinoid receptors, as both drugs were complete inhibitors of [$^3$H]SR141716A binding in mouse brain membrane preparations under [$^{35}$S]GTPγS assay buffer conditions (data not shown). These data are the first demonstration that $\Delta^9$-tetrahydrocannabinol stimulates [$^{35}$S]GTPγS binding via cannabinoid receptors and is a partial agonist in mouse brain preparations.

The cerebellum expresses the highest levels of cannabinoid receptor binding in the brain (Rinaldi-Carmona et al., 1996), thus it should be an excellent tissue to study G protein activation by cannabinoids. However, $\Delta^9$-tetrahydrocannabinol did not reproducibly stimulate [$^{35}$S]GTPγS binding in membranes isolated from the rat cerebellum (Selley et al., 1996). As these investigators used similar experimental methods to our own, we attribute these contradictory findings to differences in the efficiency of receptor coupling to G protein between whole brain and cerebellar membranes upon $\Delta^9$-tetrahydrocannabinol stimulation or to species differences. Our finding that $\Delta^9$-tetrahydrocannabinol is a partial agonist in our assay is consistent with an earlier report demonstrating that $\Delta^9$-tetrahydrocannabinol did not maximally inhibit forskolin-stimulated cAMP levels in cannabinoid $CB_1$ receptor transfected CHO-K1 cells (Matsuda et al., 1990).

**Note added in proof:** After acceptance of this manuscript we became aware of a recent report (Sim et al., 1996) that concluded that $\Delta^9$-tetrahydrocannabinol is a partial agonist in rat cerebellar membranes.

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