ELSEVIER

European Journal of Pharmacology 321 (1997) R1–R3

ejp

Rapid communication

AM630 antagonism of cannabinoid-stimulated [^{35}S]GTPγS binding in the mouse brain

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Received 6 January 1997; accepted 8 January 1997

Abstract

This research was designed to determine the action of the novel aminoalkylindole AM630 (6-iodo-pravadoline) at the cannabinoid receptor by studying its interaction with the cannabinoid receptor agonist WIN 55,212-2 (*R*(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-*de*]-1,4-benzoxazin-y1]-(1-naphthalenyl)methanone mesylate) on guanosine-5′-*O*-(3-[^{35}S]thio)triphosphate ([^{35}S]GTPγS) binding in mouse brain. WIN 55,212-2 stimulated [^{35}S]GTPγS binding, while AM630 had no effect. AM630 antagonized WIN 55,212-2-induced [^{35}S]GTPγS binding and shifted the WIN 55,212-2 dose–response curve to the right. These results clearly demonstrate that AM630 exerts cannabinoid receptor antagonist properties in the brain.

Keywords: AM630; Cannabinoid receptor antagonist; [^{35}S]GTPγS binding

Our knowledge of receptor pharmacology is greatly facilitated by the development of specific receptor blockers and demonstration of selective antagonism in whole-animal, cellular and molecular models of drug action. The novel aminoalkylindole AM630 (6-iodo-pravadoline) was originally found to attenuate the effects of cannabinoids in the mouse vas deferens (Pertwee et al., 1995). More recently, however, the same laboratory has reported that AM630 may exert cannabinoid receptor agonist properties in the isolated guinea-pig myenteric plexus-longitudinal muscle preparation (Pertwee et al., 1996) and to exert neither agonist nor antagonist activity in the isolated mouse urinary bladder (Pertwee and Fernando, 1996).

Because many cannabinoid effects of potential clinical interest are centrally mediated, this study was designed to determine whether AM630 is a cannabinoid receptor agonist or antagonist in the brain by studying its influence on guanosine-5′-*O*-(3-[^{35}S]thio)triphosphate ([^{35}S]GTPγS) binding, which is mediated by cannabinoid receptors in the brain (Sim et al., 1995; Selley et al., 1996) as well as its interaction with the cannabinoid receptor agonist WIN 55,212-2 (*R*(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)-methyl]pyrrolo[1,2,3-*de*]-1,4-benzoxazin-yl]-(1-naphthalenyl)methanone mesylate).

Male (Institute for Cancer Research, ICR) mice, weighing 20–30 g (Harlan Sprague-Dawley, Indianapolis, IN, USA) were killed by cervical dislocation. Whole mouse brains were removed and homogenized in 20 volumes of ice-cold TE buffer (10 mM Tris-HCl, 1.0 mM EDTA, pH 7.4). The homogenate was centrifuged at 40 000 × *g* at 4°C for 15 min, and the pellet was resuspended in 20 volumes of assay buffer (25 mM Tris-HCl, 150 mM NaCl, 2.5 mM $MgCl_2$, 1.0 mM EDTA, 50 μM GDP, 30 μM bestatin, 10 μM captopril and 0.1 mM phenylmethylsulfonyl fluoride, pH 7.4). Following a preincubation at 30°C for 30 min, membranes were centrifuged then resuspended in fresh assay buffer to an optical density at 280 nm

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0014-2999/97/$17.00
PII S0014-2999(97)00047-2

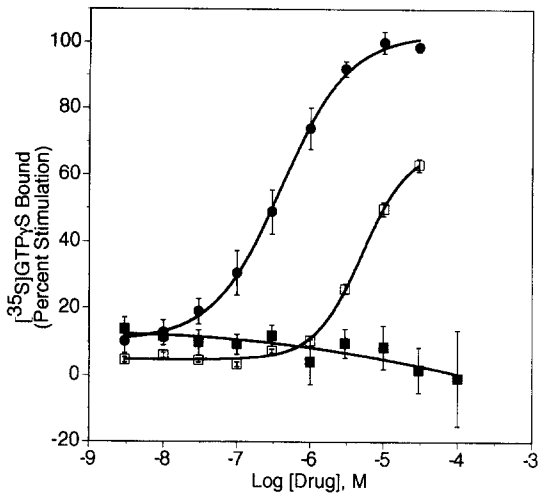

Fig. 1. Effect of various cannabinoid agents on [^{35}S]GTPγS binding to membranes prepared from mouse whole brain. Membranes were incubated with [^{35}S]GTPγS in the presence of WIN 55,212-2 (●, $n=9$); WIN 55,212-2 and AM630 (□, $n=3$); and AM630 (■, $n=4$). Each symbol represents the mean response and the vertical lines indicate the S.E.M. expressed as percent stimulation of [^{35}S]GTPγS binding using the maximum stimulation by WIN 55,212-2 as 100%. Basal [^{35}S]GTPγS binding in the presence of GDP (50 μM) was 17.85±2.63 fmol/mg protein for WIN 55,212-2, 15.68±4.29 fmol/mg protein for AM630, and 21.87±0.72 fmol/mg protein for AM630+WIN 55,212-2; there was no statistically significant difference between any of these groups (one-way ANOVA). The formula, K_e = [antagonist]/[dose ratio − 1], was used to determine the K_e value for AM630 (K_e = 3.1 μM; [antagonist] is the concentration of antagonist, and [dose ratio] is the ratio of EC_{50} values in the presence and absence of the antagonist.

(OD_{280}) of 0.8. Membranes were incubated with appropriate concentrations of WIN 55,212-2 and/or AM630 in the presence of 0.1 nM [^{35}S]GTPγS in a total volume of 1.0 ml. After 90 min incubation at 30°C, the reaction was terminated by rapid filtration under vacuum through Whatman GF/B glass fiber filters, followed by four washes with 4 ml ice-cold 25 mM Tris/120 mM NaCl, pH 7.4. Bound radioactivity was measured by liquid scintillation spectrophotometry after an overnight extraction with Eco-Lite scintillation cocktail.

WIN 55,212-2 (Research Biochemicals International, Natick, MA, USA) and AM630 (synthesized in Dr. Makriyannis' laboratory) were initially dissolved in 50% ethanol or 50% dimethyl sulfoxide (DMSO) then further diluted with distilled water. The highest final concentration of ethanol or DMSO in assay samples was determined in preliminary studies to have no effect on [^{35}S]GTPγS binding.

Fig. 1 shows dose–response curves for WIN 55,212-2-induced stimulation of [^{35}S]GTPγS binding in mouse brain in the absence and presence of AM630. WIN 55,212-2 elevated [^{35}S]GTPγS binding, producing a sigmoidal dose–response curve; however, in the presence of 100 μM AM630, the WIN 55,212-2 dose–response curve was shifted to the right. On the other hand, AM630 had no appreciable effect on [^{35}S]GTPγS binding up to 100 μM.

The availability of pharmacological antagonists is a key contributor to identification of receptor involvement in any physiological or pharmacological function. The first cannabinoid receptor antagonist, SR 141,716A (Rinaldi-Carmona et al., 1994), competitively antagonized cannabinoid-induced inhibition of electrically evoked contractions of isolated smooth muscle (Pertwee et al., 1995, 1996; Pertwee and Fernando, 1996) and cannabinoid-induced stimulation of [^{35}S]GTPγS binding (Sim et al., 1995; Selley et al., 1996).

AM630 was recently introduced as a competitive antagonist at cannabinoid receptors in the isolated mouse vas deferens (Pertwee et al., 1995). Increasing concentrations of AM630 shifted progressively to the right the dose–response curves for a host of cannabinoid receptor agonists, including WIN 55,212-2, CP 55,940 and anandamide, in inhibition of electrically evoked twitches of the vas deferens (Pertwee et al., 1995). However, a subsequent study demonstrated that AM630 alone inhibited electrically evoked contractions of the isolated guinea pig myenteric plexus-longitudinal muscle with an IC_{50} of 1.9 μM; this apparent cannabimimetic effect of AM630 was blocked by SR 141,716A (Pertwee et al., 1996). More recently, AM630 reportedly failed to antagonize cannabinoid-induced inhibition of electrically evoked contractions of the mouse urinary bladder; moreover, concentrations of AM630 as high as 3.16 μM failed to influence urinary bladder contraction (Pertwee and Fernando, 1996).

The results of this study clearly demonstrate that AM630 can antagonize WIN 55,212-2-induced stimulation of [^{35}S]GTPγS binding in the mouse brain. The K_e value for AM630 was calculated to be 3.1 μM (Tallarida and Murray, 1987). It is concluded that AM630 exerts cannabinoid receptor antagonist properties in the central nervous system.

Acknowledgements

This research was supported in part by grants from the Arizona Disease Control Research Commission and N.I.D.A.

References

Pertwee, R.G., Fernando, S.R., 1996. Evidence for the presence of cannabinoid CB1 receptors in mouse urinary bladder. Br. J. Pharmacol. 118, 2053–2058.

Pertwee, R.G., Griffin, G., Fernando, S., Li, X., Hill, A., Makriyannis, A., 1995. AM630, a competitive cannabinoid receptor antagonist. Life Sci. 56, 1949–1955.

Pertwee, R.G., Fernando, S.R., Nash, J.E., Coutts, A.A., 1996. Further evidence for the presence of cannabinoid CB1 receptors in guinea-pig small intestine. Br. J. Pharmacol. 118, 2199–2205.

Rinaldi-Carmona, M., Barth, F., Heaulme, M., Shire, D., Calandra, B., Conghy, C., Martinez, S., Maruani, J., Neliat, G., Caput, D., Ferrara,

P., Soubrie, P., Breliere, J.C., LeFur, G., 1994. SR 141.716A, a potent and selective antagonist of the brain cannabinoid receptor. FEBS Lett. 350, 240–244.

Selley, D.E., Stark, S., Sim, L.J., Childers, S.R., 1996. Cannabinoid receptor stimulation of guanosine-5′-*O*-(3-[^{35}S]thio)triphosphate binding in rat brain membranes. Life Sci. 59, 659–668.

Sim, L.J., Selley, D.E., Childers, S.R., 1995. In vitro autoradiography of receptor-activated G proteins in rat brain by agonist-stimulated guanylyl 5′-[γ[^{35}S]thio]-triphosphate binding. Proc. Natl. Acad. Sci. USA 92, 7242–7246.

Tallarida, R.J., Murray, R.B, 1987. Manual of Pharmacologic Calculations with Computer Programs, 2nd ed., Springer, New York, NY.