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AM630 IS A COMPETITIVE CANNABINOID RECEPTOR ANTAGONIST IN THE GUINEA PIG BRAIN

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ABSTRACT AM630 has been demonstrated to be a cannabinoid receptor antagonist in the mouse brain and vas deferens. Conversely, it was recently reported that AM630 acts as a cannabinoid agonist in the guinea pig ileum. This research was designed to determine whether the difference in the action of AM630 is species specific. Studies conducted in guinea pig brain reveal that AM630 antagonizes the stimulatory effect of the cannabinoid agonist WIN 55,212-2 on [^{35}S]GTPγS binding suggesting that difference in AM630 activity in different tissues is not due to species variation.

Key Words: cannabinoid antagonist, [^{35}S]GTPγS binding, guinea pig

Introduction

The novel aminoalkylindole 6-iodo-pravadoline (AM630) was first reported to attenuate the effects of cannabinoids in the mouse vas deferens (1). Recent work from our laboratory also demonstrated that AM630 antagonized the ability of the cannabinoid agonist WIN 55,212-2 (R(+)-[2,3-dihydro-5-methyl-3-[(monopholinyl) methyl]pyrrolo[1,2,3-de]-1,4-benzoxan-yl]-(1-naphathalenyl)methanone mesylate) to stimulate guanosine-5'-O-(3-[^{35}S]thio)triphosphate ([^{35}S]GTPγS) binding in mouse brain membrane preparations (2). On the other hand, AM630 was reported to act as a cannabinoid receptor agonist in the isolated guinea pig myenteric plexus-longitudinal muscle preparation (3). The present study was conducted to determine whether this discrepancy might be due to a species-dependent difference in the actions of AM630. Accordingly, we measured the actions of AM630 on [^{35}S]GTPγS binding in guinea pig brain.

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Methods

Male Hartley guinea pigs, weighing 150-170 g (Harlan Sprague Dawley, Indianapolis, Indiana) were euthanized by decapitation. Whole brains were immediately removed and homogenized in 20 volumes of ice-cold TE buffer (10 mM Tris-HCl, 1.0 mM EDTA, pH 7.4). In some cases, brain membranes were prepared and frozen at -70°C and used within a 3-week period. The homogenate was centrifuged at 48,000 X 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). The membranes were then preincubated for 30 min at 30°C to remove endogenous cannabinoids. Following centrifugation, the membranes were resuspended in fresh assay buffer to an optical $density_{280}$ (OD_{280}) of 0.8.

Membranes were incubated with increasing concentrations of WIN 55,212-2 (Research Biochemicals International, Natick, Massachusetts) and/or AM630 (synthesized in Dr. Makriyannis' laboratory) in the presence of 0.1 nM [^{35}S]GTPγS (1,250 Ci/mmol, New England Nuclear, Boşton, Massachusetts) 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. Filters were removed

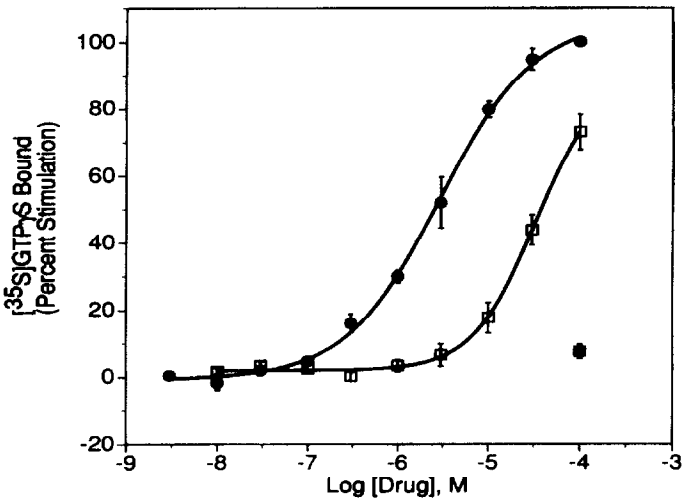

Fig. 1

Fig. 1. Effect of WIN 55,212-2 and AM630 on [^{35}S]GTPγS binding to membranes prepared from guinea pig whole brain. Membranes were incubated with [^{35}S]GTPγS in the presence of: solid circles, WIN 55,212-2 (n = 3); open squares, WIN 55,212-2 and AM630 (n = 3); and solid squares, AM630 (100 μM) (n = 3). 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 26.9 ± 2.66 fmol/mg protein for the AM630 alone and WIN 55,212-2 groups, and 30.5 ± 2.91 fmol/mg protein for AM630 + WIN 55,212-2 group; there was no statistically significant difference between these means (one way ANOVA). The formula, K_B = [antagonist]/[dose ratio - 1], was used to determine the dissociation constant for the antagonist AM 630 (K_B = 9.3 μM). The dose ratio is the ratio of EC_{50} values in the presence and absence of the antagonist.

and extracted in 10 ml EcoLite® scintillation cocktail at 4°C for at least 6 hr before bound radioactivity was measured by liquid scintillation spectrophotometry.

WIN 55,212-2 and AM630 were prepared as 1 mM using 33.3 % and 55 % ethanol, respectively. Further dilutions were prepared using distilled water.

Results

Fig. 1 shows dose-response curves for WIN 55,212-2-induced stimulation of [^{35}S]GTPγS binding in guinea pig brain in the absence and presence of AM630. WIN 55,212-2 produced a concentration-dependent increase in [^{35}S]GTPγS binding. In the presence of AM630 (100 μM), the WIN 55,212-2 dose-response curve was shifted to higher concentrations of drug. On the other hand, AM630 (100 μM) produced a negligible 7.5% increase in [^{35}S]GTPγS binding above basal levels.

The EC_{50} values for WIN-55,212-2-induced stimulation of [^{35}S]GTPγS binding were 2.98 μM and 35.1 μM in the absence and presence of AM630, respectively. Therefore, the rightward shift in the dose-response curve for WIN-55,212-2 in the presence of AM630 reflects an 11.8-fold increase in the EC_{50} of WIN-55,212-2. The dissociation constant (K_B value) (4) of AM630 was 9.3 μM.

Discussion

AM630 was originally designed to be an antagonist at the cannabinoid receptor (1). In the isolated mouse vas deferens, AM630 caused a concentration-related rightward shift in the dose-response curves for the cannabinoid agonists WIN 55,212-2, CP 55,940 and anandamide in inhibition of electrically-evoked contractions (1). In addition, we showed that AM630 antagonized WIN 55,212-2-induced stimulation of [^{35}S]GTPγS binding in the mouse brain (2). However, these reports are inconsistent with results of another study (3), in which AM630 was shown to exert a cannabinoid agonist action. AM630 reportedly caused inhibition of electrically-evoked contractions of the isolated guinea pig myenteric plexus-longitudinal muscle; this apparent cannabimimetic effect of AM630 was blocked by SR 141,716A, a CB1 antagonist (5).

One possible explanation for this discrepancy in AM630 action would be a species difference. Since stimulation of [^{35}S]GTPγS binding appears to be a sensitive test of cannabinoid agonistic activity (6), the present study measured changes in [^{35}S]GTPγS binding in membranes prepared from guinea pig brain in order to reveal whether AM630 exerts agonistic or antagonist properties at the cannabinoid receptor. The results of this study show AM630 alone had no appreciable effect on [^{35}S]GTPγS binding in guinea pig. In contrast, AM630 antagonized WIN 55,212-2 stimulated [^{35}S]GTPγS binding as reflected by the rightward shift in the WIN 55,212-2 dose-response curve in the presence of AM630. These findings clearly demonstrate that AM630 is an antagonist in the guinea pig brain and suggest that the reported agonistic effect of AM630 in the guinea pig myenteric plexus-longitudinal muscle is not the result of a species difference between the guinea pig and the mouse.

A second possible explanation for the discrepancy in AM630 action is cannabinoid receptor types. Since AM630 was an antagonist in the mouse vas deferens (1) and the mouse brain (2), it is possible that AM630 acts as an agonist at a different cannabinoid receptor in the guinea pig myenteric plexus-longitudinal muscle. The precedent for such a situation lies in the dual actions of β-flunaltrexamine as both an agonist at kappa-opioid receptors and an antagonist at mu-opioid receptors (7,8).

Yet another possible explanation for the discrepancy in AM630 action is the role of the tissue in determining drug action. The agonist and antagonist actions of AM630 in guinea pig myenteric plexus-longitudinal muscle and brain, respectively, may be determined by differences in levels of cannabinoid receptor expression, G-protein-coupling or second messenger pathways. However, the mechanism(s) that allow AM630 to be an agonist or antagonist, depending on the tissue studied, remain(s) to be elucidated.

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