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Examination of the effect of the cannabinoid receptor agonist, CP 55,940, on electrically evoked transmitter release from rat brain slices

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Abstract

In the present study we examined the effect of the cannabinoid receptor agonist, {[1a,2-(*R*)-5-(1,1-dimethylheptyl)-2-[5-hydroxy-2-(3-hydroxypropyl)cyclohexyl]-phenol; CP 55,940} on [^{14}C]acetylcholine and [^{3}H]norepinephrine release from hippocampal slices and on [^{14}C]acetylcholine release from striatal slices. CP 55,940 potently inhibited electrically evoked [^{14}C]acetylcholine release from hippocampal slices, with an EC_{50} of 0.02 μM and a maximal inhibition of 61% at 1 μM. The inhibition of acetylcholine release by CP 55,940 was partially antagonized (60%) by the cannabinoid receptor antagonist, {[*N*-piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride; SR 141716A}. Alone, SR 141716A significantly enhanced stimulated [^{14}C]acetylcholine release. In contrast to the effects of CP 55,940 on [^{14}C]acetylcholine release, electrically evoked [^{3}H]norepinephrine release from hippocampal slices and [^{14}C]acetylcholine release from striatal slices were both unaffected by this compound. Similarly, hippocampal [^{3}H]norepinephrine release and striatal [^{14}C]acetylcholine release were not affected by SR 141716A. In conclusion, the results of this study extend our previous data indicating that cannabinoid receptors modulate acetylcholine release in the hippocampus. The effects of cannabinoid receptor activation on [^{3}H]acetylcholine release in the hippocampus does not appear to extend to [^{3}H]norepinephrine release from this region or to acetylcholine release from the striatum. © 1997 Elsevier Science B.V. All rights reserved.

Keywords: Cannabinoid receptor ; CP 55,940; SR 141716A; Brain slice; Acetylcholine

1. Introduction

Cannabinoid receptors are G-protein-coupled receptors that are linked to the inhibition of adenylate cyclase (Howlett et al., 1988). Two types of cannabinoid receptor, designated the cannabinoid CB_1 and CB_2 receptors, have been identified and cloned (Matsuda et al., 1990; Munro et al., 1993). The cannabinoid CB_1 receptor is located both in the central nervous system (Herkenham et al., 1990) and in some peripheral tissues such as the testis (Pacheco et al., 1991), ileum (Pertwee et al., 1992) and uterus (Das et al., 1995). The cannabinoid CB_2 receptor has been identified in macrophages and in the spleen (Munro et al., 1993).

The screening of brain extracts for binding activity at the cannabinoid receptor has identified an arachidonic acid derivative, termed anandamide, which binds with moderately high affinity to these receptors (Devane et al., 1992). This compound appears to be a partial agonist (Mackie et al., 1993), at cannabinoid CB_1 receptors. Anandamide, along with other related compounds (Priller et al., 1995), has been proposed to be an endogenous transmitter for cannabinoid receptors.

A number of synthetic cannabinoid receptor agonists have been developed with an affinity for the cannabinoid receptors greater than that of Δ-9-tetrahydrocannabinol (Devane et al., 1988; Howlett et al., 1988). The most potent of these has been {[1a,2-(*R*)-5-(1,1-dimethylheptyl)-2-[5-hydroxy-2-(3-hydroxy-propyl)cyclohexyl]-phenol; CP 55,940} (Herkenham et al., 1990). In addition to the synthetic cannabinoid receptor agonists, an unrelated class of compounds that are agonists at the cannabinoid receptor, are aminoalkyindoles such as {*R*-(+)-(2,3-dihydro-5-methyl-3-[{4-morpholinyl}methyl]pyrol[1,2,3-de]-1,4-benzozoxazin-6-yl)(naphthalenyl)methanone monomethanesulfate; WIN 55212-2} (Compton et al., 1992). These compounds were based on the structure of pravadoline, which was first developed as a cyclooxygenase inhibitor for use as an antinociceptive agent, but was also found to have an

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action on cannabinoid receptors (Compton et al., 1992; D'Ambra et al., 1992). More recently, a synthetic cannabinoid receptor antagonist, {[*N*-piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride; SR 141716A}, has been developed (Rinaldi-Carmona et al., 1994, 1995). This compound is structurally distinct from both the synthetic cannabinoids and the aminoalkylindole cannabinoid compounds.

Despite their abundance in the central nervous system, the function of these receptors is not clear. Electrophysiological studies on cultured neurons have demonstrated that cannabinoid receptor activation inhibits calcium channels (Mackie and Hille, 1992) and opens potassium channels (Deadwyler et al., 1995; Henry and Chavkin, 1995). If cannabinoid receptors have this same action on calcium and potassium channels on the synaptic terminals then these receptors may function to inhibit neurotransmitter release. This is supported by recent studies reporting an inhibitory effect of cannabinoids on norepinephrine release in the mouse vas deferens (Ishac et al., 1996), acetylcholine release in the guinea-pig small intestine (Pertwee et al., 1996) and glutamate release in cultured hippocampal cells (Shen et al., 1996). We have recently reported that WIN 55212-2 produces a strong inhibition of electrically evoked [^{14}C]acetylcholine release from hippocampal slices (Gifford and Ashby, 1996), an area with a high density of cannabinoid receptors (Herkenham et al., 1991a). In addition, SR 141716A alone produced a substantial enhancement in the evoked [^{14}C]acetylcholine release from this region (Gifford and Ashby, 1996). This latter finding suggests either that cannabinoid receptors are constitutively active and produce a tonic inhibition of acetylcholine release or that SR 141716A is antagonizing the effects of an endogenous cannabinoid agonist that is released along with acetylcholine.

The objectives of the present study were firstly to determine whether our original observations on the effects of WIN 55212-2 on hippocampal [^{14}C]acetylcholine release could be reproduced with the synthetic cannabinoid agonist, CP 55,940, and secondly to determine whether this compound also affects the release of other neurotransmitters. For the latter objective, we examined the effects of CP 55,940 and SR 141716A on [^{3}H]norepinephrine release from hippocampal slices and on [^{14}C]acetylcholine release from striatal slices. The striatum was chosen since, like the hippocampus, it also has a high density of cannabinoid receptors and functional brain slices can be readily prepared from this region.

2. Materials and methods

2.1. Materials

CP 55,940 was obtained from Pfizer (Groton, CT, USA) and SR 141716A from Sanofi Recherche (Montpellier, France). [^{14}C]acetylcholine (55 mCi/mmol) and [^{3}H]norepinephrine (15 Ci/mmol) were purchased from Amersham (Amersham, UK) and Dupont NEN (Wilmington, DE, USA), respectively.

2.2. Superfusion procedure

Male Sprague-Dawley rats (200–350 g, Taconic, Germantown, NY, USA) were decapitated, their brains quickly removed and the hippocampus or striatum dissected out on an ice-cold aluminum block. Following dissection, 300 μm tissue slices were cut with a vibratome and the slices transferred to 2 ml of Krebs buffer (119.5 mM NaCl, 3.3 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 11 mM glucose, 0.03 mM EDTA, 0.6 mM ascorbate, pH 7.4), saturated with 95% O_2/5% CO_2, and containing 18 μM [^{14}C]choline or 0.04 μM [^{3}H]norepinephrine. Following incubation with the radiolabel for 15 min at 21°C, the slices were transferred to eight superfusion chambers (two to three slices per chamber). The slices were sandwiched between wire mesh screens positioned midway between two platinum electrodes connected to the stimulation apparatus. Slices were superfused at 37°C, at a rate of 0.5 ml/min, with oxygenated Krebs buffer containing 10 μM hemicholinium-3, to prevent reuptake of [^{14}C]choline hydrolyzed from released [^{14}C]acetylcholine. Six-min fractions were collected beginning 120 min after the start of superfusion until the termination of the experiment.

After the end of the superfusion, the slices were removed from the chambers and their residual radioactivity measured. To ensure removal of drug solutions from the apparatus, the superfusion chambers were washed with detergent overnight and all plastic tubing replaced before starting another experiment.

2.3. Times of drug additions and tissue stimulation

The tissue slices were subjected to three periods of electrical stimulation (*S*1, *S*2 and *S*3), each of 3 min duration, beginning 115 min, 160 min and 231 min after superfusion was started. Each stimulation period consisted of a train of unipolar pulses (20 mA, 2 ms) at a rate of 0.5 s^{-1}.

CP 55,940 and SR 141716A were dissolved in 40% cyclodextrin (w/v) or 100% dimethyl sulfoxide, respectively, and added to the superfusion medium. The maximal final concentrations of the betacyclodextrin or dimethyl sulfoxide vehicles in the superfusion medium were less than 0.02% and 0.05%, respectively. Previous experiments suggested that these concentrations of vehicle did not affect electrically evoked [^{14}C]acetylcholine release (Gifford and Ashby, 1996)

To allow time for equilibration in the slice CP 55,940 was added to the superfusion medium 60 min before

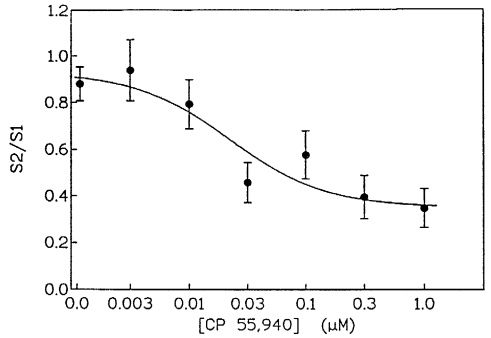

Fig. 1. Effect of CP 55,940 on the electrically evoked release of [^{14}C]acetylcholine from hippocampal slices. $S3/S2$ ratio represents the release in the presence of CP 55,940 ($S3$) to that in its absence ($S2$). Data are the means (±S.E.M.) of 7–12 determinations.

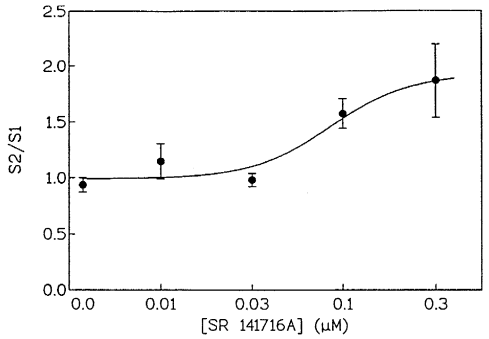

Fig. 3. Effect of SR 141716A alone on the electrically evoked release of [^{14}C]acetylcholine from hippocampal slices. $S2/S1$ ratio represents the release in the presence of SR 141716A ($S2$) to that in its absence ($S1$). Data are the means (±S.E.M.) of 5–7 determinations.

stimulation ($S2$ or $S3$) and SR 141716A 35 min before stimulation ($S2$). Both ligands were kept in the medium until the end of the experiment.

2.4. Data analysis

Stimulation-evoked release ($S1$, $S2$ and $S3$) was calculated by subtracting the counts in the fraction collected immediately before initiating stimulation (i.e., basal release) from those in the two fractions collected immediately after initiating stimulation. The effect of CP 55,940 or SR 141716A on stimulation-evoked release was determined by calculating the amount of evoked release of radioactivity before adding drug relative to the amount of evoked release after adding drug (i.e., $S2/S1$ or $S3/S2$). Calculating the data as $S2/S1$ or $S3/S2$ ratios compensates for variations between chambers in the amount of stimulation-evoked release. Stimulation-evoked release for $S1$ (i.e., before drugs) ranged from 0–244 cpm for hippocampal [^{14}C]acetylcholine release, 0–485 cpm for hippocampal [^{3}H]norepinephrine release and 33–4565 cpm for striatal [^{14}C]acetylcholine release. To avoid inaccurate $S2/S1$ or $S3/S2$ ratios from those slices showing relatively little stimulation-evoked overflow, slices having an evoked release before drug addition of < 40 cpm for [^{14}C]acetylcholine or < 100 cpm for [^{3}H]norepinephrine were excluded from the data analysis.

The effects of CP 55,940 and SR 141716A on the $S2/S1$ ratios in Tables 1 and 2 were analyzed using a single factor analysis of variance, followed by a Dunnett's test (two-tailed) for comparing treatment means to a single control mean.

The fitted curves in Figs. 1–3 and the EC_{50} for the effect of CP 55,940 were calculated using the non-linear regression program contained in Graphpad Inplot (Graphpad Software).

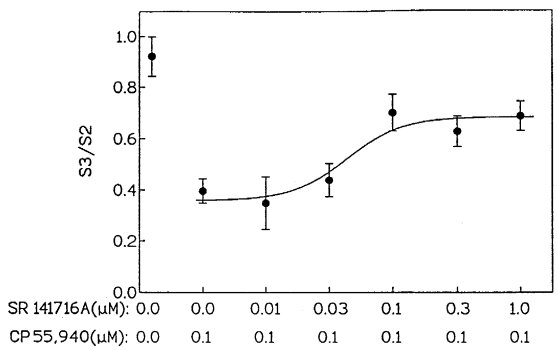

Fig. 2. Antagonism by SR 141716A of the inhibition of electrically evoked release of [^{14}C]acetylcholine by CP 55,940 (0.3 μM) in hippocampal slices. CP 55,940 was added prior to $S3$, while SR 141716A was added prior to $S2$. Data are the means (±S.E.M.) of 6–14 determinations.

3. Results

3.1. Effect of CP 55,940 on hippocampal [^{14}C]acetylcholine release

In the hippocampus, CP 55,940 produced a dose-dependent inhibition of electrically evoked [^{14}C]acetylcholine release (Fig. 1). The maximum inhibition of [^{14}C]acetylcholine release by CP 55,940 was 61% and the EC_{50} for CP 55,940 in producing half of its maximal

Table 1
Effect of CP 55,940 and SR 141716A on electrically stimulated [^{3}H]norepinephrine release from hippocampal slices

	$S2/S1$ ratio
Control	0.92 ± 0.06 (24)
0.1 μM CP 55,940	0.82 ± 0.12 (9)^{NS}
0.3 μM CP 55,940	0.85 ± 0.26 (7)^{NS}
1.0 μM CP 55,940	1.05 ± 0.20 (6)^{NS}
0.1 μM SR 141716A	0.77 ± 0.12 (3)^{NS}
0.3 μM SR 141716A	1.13 ± 0.10 (5)^{NS}

Data are means (±S.E.M.) of the number of determinations indicated. NS, not significantly different from control (two-tailed Dunnett's test).

Table 2
Effect of CP 55,940 and SR 141716A on electrically stimulated [^{14}C]acetylcholine release from striatal slices

	S2/S1 ratio
Control	0.85 ± 0.10 (9)
0.3 μM CP 55,940	0.98 ± 0.14 (5)
1.0 μM CP 55,940	0.75 ± 0.09 (6)
0.3 μM SR 141716A	0.86 ± 0.10 (4)
1.0 μM SR 141716A	0.82 ± 0.12 (9)

Data are means (± S.E.M.) of the number of determinations indicated. Effects of CP 55,940 and SR 141716A were not significantly different from control (two-tailed Dunnett's test).

effect was 22 nM. Basal [^{14}C]acetylcholine release was unaffected by CP 55,940 (data not shown).

3.2. Effect of SR 141716A on hippocampal [^{14}C]acetylcholine release

The inhibition of [^{14}C]acetylcholine release by 0.1 μM CP 55,940 was dose dependently antagonized by SR 141716A (Fig. 2). However, SR 141716A did not produce a complete reversal of the effects of CP 55,940, but instead inhibited the effects of CP 55,940 by a maximum of approximately 60%.

The addition of SR 141716A alone resulted in an up to two-fold potentiation of electrically evoked [^{14}C]acetylcholine release (Fig. 3). This potentiating effect of SR 141716A was also observed in our previous study (Gifford and Ashby, 1996). Basal [^{14}C]acetylcholine was unaffected by SR 141716A (data not shown).

3.3. Effect of CP 55,940 and SR 141716A on hippocampal [^{3}H]norepinephrine release and striatal [^{14}C]acetylcholine release.

CP 55,940 did not appear to significantly inhibit electrically evoked hippocampal [^{3}H]norepinephrine release (Table 1) or striatal [^{14}C]acetylcholine release (Table 2). The release of these transmitters was also not potentiated by SR 141716A.

4. Discussion

4.1. Effect of CP 55,940 on stimulation-evoked transmitter release.

The inhibition of electrically evoked release of [^{3}H]acetylcholine by CP 55,940 supports our previous observations, obtained using the aminoalkylindole, WIN 55,212-2, suggesting that cannabinoid receptors have a role in controlling the release of this neurotransmitter in this region (Gifford and Ashby, 1996). Lesioning studies in animals and observations in humans on the effects of muscarinic antagonists have shown that the hippocampal cholinergic system is necessary for short-term memory (Bolhuis et al., 1988; Zola-Morgan and Squire, 1993). Thus, it is possible that our observations demonstrating an inhibition of cholinergic transmission in the hippocampus by cannabinoid receptor activation may explain the memory impairments caused by marijuana smoking in humans (Schwartz et al., 1989).

The cholinergic innervation of the hippocampus is supplied by neurons whose cell bodies are located in the region of the medial septum and diagonal band of Broca (Meibach and Siegel, 1977; Lynch et al., 1978). Autoradiograms of cannabinoid receptor mRNA distribution show a relatively high level of cannabinoid receptor mRNA in this area (Mailleux and Vanderhaeghen, 1992), supporting a presynaptic localization of cannabinoid receptors on the cholinergic nerve terminals in the hippocampus. However, since the hippocampus itself also has a high level of cannabinoid mRNA, the possibility cannot be excluded that the effect of CP 55,940 on [^{3}H]acetylcholine release was mediated via the enhancement of the stimulation-evoked release of another neurotransmitter which subsequently inhibited the release of acetylcholine. However, in preliminary studies we did not observe any reduction in the inhibition of hippocampal [^{14}C]acetylcholine release by CP 55,940 by the 5-HT$_{1A,B}$ receptor antagonist, pindolol, the 5-HT$_{2A,C}$ and D$_2$ receptor antagonist, ritanserin or by the non-selective opioid receptor antagonist, naloxone (unpublished observations).

In the present study, CP 55,940 had no significant effect on [^{3}H]norepinephrine release from the hippocampus or [^{14}C]acetylcholine release from the striatum. In the latter area, the absence of an effect of CP 55,940 was unexpected since in addition to a dense cholinergic innervation this region has a density of cannabinoid receptors similar to that of the hippocampus (Herkenham et al., 1991a,b). The cholinergic innervation of the striatum differs from that of the hippocampus in that it is supplied by local interneurons rather than from the basal cholinergic forebrain system that supplies the hippocampus (Butcher and Woolf, 1984). It may be that cannabinoid effects on acetylcholine release are confined to cells in the latter system. However, it is of interest that in the guinea-pig small intestine preparation, cannabinoid receptor agonists will similarly inhibit electrically evoked acetylcholine release (Pertwee et al., 1996).

4.2. Effect of SR 141716A on stimulation-evoked transmitter release.

A number of studies have shown that SR 141716A can effectively antagonize both the biochemical and behavioral effects of cannabinoid agonists (Rinaldi-Carmona et al., 1994, 1995; Compton et al., 1996). In the current study, we observed that this compound was able to antagonize, albeit incompletely, the effect of CP 55,940 on [^{14}C]acetylcholine release. The incomplete blockade of the

effects of CP 55,940 by SR 141716A may suggest that in addition to acting on the CB_1 receptor, CP 55,940 also inhibits [^{14}C]acetylcholine release via a receptor that is insensitive to SR 141716A. One candidate for this would be the cannabinoid CB_2 receptor. The presence of this receptor in the central nervous system has recently been demonstrated (Skaper et al., 1996).

In addition to antagonizing the effects of CP 55,940, SR 141716A alone produced a substantial enhancement of electrically evoked acetylcholine release. The potentiation of [^{14}C]acetylcholine release by SR 141716A alone was also observed in our previous study (Gifford and Ashby, 1996). The absence of a similar effect of SR 141716A on [^{3}H]norepinephrine release in the hippocampus and [^{3}H]acetylcholine release in the striatum suggests that the potentiation of [^{14}C]acetylcholine release in the hippocampus is due to a specific effect of SR 141716A on cannabinoid receptors. A potentiation of electrically evoked acetylcholine release by SR 141716A alone was also reported by Pertwee et al. (1996) in the guinea-pig small intestine preparation. Two explanations can be proposed to account for this action of SR 141716A in both our own study and in that of Pertwee et al. (1996). First, SR 141716A may be acting as an inverse agonist on cannabinoid receptors. This would imply that these receptors are constitutively active and produce a tonic suppression of acetylcholine release. This is unlikely as the systemic administration of SR 141716A does not elicit behaviors produced by cannabinoid receptor agonists (Ashby et al., unpublished observations); in fact, it has reverse cannabimimetic actions (Rinaldi-Carmona et al., 1994). Second, SR 141716A may be antagonizing the actions of an endogenous cannabinoid compound (possibly anandamide) that is also released in the slices by electrical stimulation along with acetylcholine.

In conclusion, our results indicate that CP 55,940, like WIN 55212-2, potently inhibits [^{14}C]acetylcholine release from hippocampal slices and this effect is incompletely antagonized by SR 141716A. Both the inhibitory effects of CP 55,940 and the potentiating effects of SR 141716A appear to be confined to hippocampus.

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