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Agonist-antagonist characterization of 6′-cyanohex-2′-yne-Δ^8-tetrahydrocannabinol in two isolated tissue preparations

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Abstract

This investigation was directed at characterizing some of the pharmacological properties of 6′-cyanohex-2′-yne-Δ^8-tetrahydrocannabinol (O-823), a compound with high affinity for cannabinoid binding sites ($K_i = 0.77$ nM). In mouse vasa deferentia, O-823 behaved as a potent partial cannabinoid CB_1 receptor agonist ($EC_{50} = 0.015$ nM). In the guinea-pig myenteric plexus preparation, it antagonized WIN 55,212-2 {(*R*)-(+)-[2,3-dihydro-5-methyl-3-[(4-morpholino)methyl]pyrrolo-[1,2,3-de]-1,4-benzoxazin-6-yl](1-naphthyl)methanone} and CP 55,940 {(−)-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-4-(3-hydroxypropyl)cyclohexan-1-ol} with K_d values of 0.65 and 0.27 nM, respectively. After in vivo Δ^9-tetrahydrocannabinol pretreatment, the sensitivity of vasa deferentia to O-823-induced inhibition of electrically evoked contractions was reduced by 127-fold. 3.162 nM O-823 was inhibitory in unpretreated vasa deferentia but antagonized CP 55,940 in pretreated tissues ($K_d = 0.26$ nM). O-823 is probably an antagonist in the myenteric plexus preparation and Δ^9-tetrahydrocannabinol pretreated vasa deferentia but a partial agonist in unpretreated vasa deferentia because the first two of these preparations contain fewer receptors than the third.

Keywords: Cannabinoid receptor antagonist; Cannabinoid receptor partial agonist; 6′-Cyanohex-2′-yne-Δ^8-tetrahydrocannabinol; O-823; SR141716A; Vas deferens, mouse; Myenteric plexus-longitudinal muscle preparation; Small intestine, guinea-pig

1. Introduction

The discovery of cannabinoid receptors has prompted the design and synthesis of many ligands for these receptors (Pertwee, 1995; Martin et al., 1995). One such compound is 6′-cyanohex-2′-yne-Δ^8-tetrahydrocannabinol (O-823), a novel analogue of the classical cannabinoid, Δ^8-tetrahydrocannabinol (Fig. 1). Initial experiments with O-823 showed it to have a high affinity for cannabinoid binding sites, prompting us to determine whether it interacts with cannabinoid receptors as an agonist or antagonist. Accordingly, further experiments were carried out to establish if O-823 shares the ability of cannabinoid receptor agonists to produce a dose-dependent inhibition of electrically evoked contractions of two isolated tissue preparations, the myenteric plexus-longitudinal muscle preparation of guinea-pig small intestine and the mouse isolated vas deferens (Pertwee et al., 1992, 1993, 1995). If O-823 did behave as an agonist, it was intended to investigate its susceptibility to antagonism by the selective cannabinoid CB_1 antagonist, SR141716A [*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride] (Rinaldi-Carmona et al., 1994). On the other hand, if O-823 was found to lack agonist activity, it was planned to explore its ability to act as a cannabinoid receptor antagonist.

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2. Materials and methods

2.1. Drugs

Δ^9-Tetrahydrocannabinol was obtained from the National Institute on Drug Abuse, CP 55,940 {(−)-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-4-(3-hydroxypropyl)-cyclohexan-1-ol} from Pfizer and WIN 55,212-2 {(*R*)-(+)-[2,3-dihydro-5-methyl-3-[(4-morpholino)methyl]pyrrolo-[1,2,3-de]-1,4-benzoxazin-6-yl](1-naphthyl)methanone} and SR141716A from Sanofi Winthrop. O-823 was synthesized in Dr. Razdan's laboratory. Drugs were mixed with 2 parts of Tween 80 by weight and dispersed in a 0.9% aqueous solution of NaCl (saline) as described previously for Δ^9-tetrahydrocannabinol (Pertwee et al., 1992). All drug additions were made in a volume of 10 μl.

2.2. In vitro binding assay

A filtration procedure was used to measure [^{3}H]CP 55,940 binding (Compton et al., 1993). This is a modification of the centrifugation method described by Devane et al. (1988). For a typical membrane preparation, five rats were decapitated and their cortices rapidly dissected free and homogenized in 30 ml of 0.32 M sucrose which contained 2 mM EDTA and 5 mM $MgCl_2$. The homogenate was centrifuged at 1600 × *g* for 10 min, and the supernatant removed. The pellet was washed twice by resuspending in 0.32 M sucrose/2 mM EDTA/5 mM $MgCl_2$ and centrifuging again as described above. The original supernatant was combined with the wash supernatants and centrifuged at 39000 × *g* for 15 min. The resulting P_2 pellet was suspended in 50 ml of buffer (50 mM Tris · HCl, pH 7.0, 2 mM EDTA, 5 mM $MgCl_2$) and incubated at 37°C for 10 min before centrifugation at 23000 × *g* for 10 min. The P_2 pellet was resuspended in 50 ml of 50 mM Tris · HCl/2 mM EDTA/5 mM $MgCl_2$ and incubated at 30°C for 10 min before centrifugation at 11000 × *g* for 15 min. The final pellet was resuspended in 10 ml of 50 mM Tris · HCl (pH 7.4) which contained 1 mM EDTA and 3 mM $MgCl_2$ and then stored at −40°C. The binding assay was performed in silanized glass tubes which contained 100 μl of radiolabelled ligand, 100 μl of competing unlabelled drug, 150 μg of membrane protein (75 μl) and sufficient buffer (50 mM Tris · HCl, pH 7.4, 1 mM EDTA, 3 mM $MgCl_2$ and 5 mg/ml bovine serum albumin) to make a final volume of 1 ml. After a 1-h incubation at 30°C, the reaction was terminated by the addition of 2 ml of ice-cold 50 mM Tris · HCl (pH 7.4) buffer containing 1 mg bovine serum albumin/ml and rapid filtration through polyethyleneimine treated Whatman GF/C glass-fibre filters. The reaction tube was washed with a 2-ml aliquot of buffer which was then also filtered. The filters were washed with two 4-ml aliquots of ice-cold buffer. They were then shaken for 60 min in 10 ml of scintillation fluid, and radioactivity quantitated by liquid scintillation spectrometry. Specific binding was typically 74% of total binding at 1 nM of [^{3}H]CP 55,940, and was defined as the difference between the binding that occurred in the presence and absence of 1 μM unlabelled CP 55,940. The displacement K_i value for O-823 was determined using the EBDA program of the KELL software package (Biosoft, Milltown, NJ, USA).

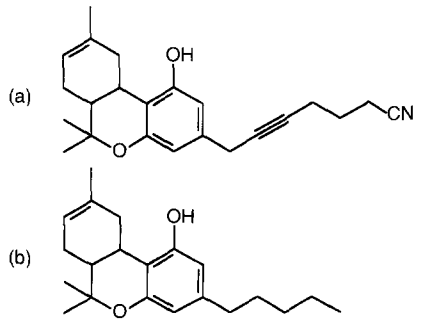

Fig. 1. Structures of (a) 6′-cyanohex-2′-yne-Δ^8-tetrahydrocannabinol (O-823) and (b) Δ^8-tetrahydrocannabinol.

2.3. In vitro experiments with isolated tissues

Experiments were performed with strips of myenteric plexus-longitudinal muscle or with isolated vasa deferentia. Each tissue was mounted in a 4 ml organ bath at an initial tension of 0.5 g using the methods described by Pertwee et al. (1992, 1995). The baths contained Krebs solution which was kept at 37°C and bubbled with 95% O_2 and 5% CO_2. The composition of the Krebs solution was (mM): NaCl 118.2, KCl 4.75, KH_2PO_4 1.19, $NaHCO_3$ 25.0, glucose 11.0 and $CaCl_2 \cdot 6H_2O$ 2.54. For experiments with the myenteric plexus preparation, this solution also contained 1.29 mM $MgSO_4 \cdot 7H_2O$. Isometric contractions were evoked by electrical field stimulation through a platinum electrode attached to the upper end and a stainless steel electrode attached to the lower end of each bath. Stimuli were generated by a Grass S48 stimulator, then amplified (Med-Lab channel attenuator) and divided to yield separate outputs to four organ baths (Med-Lab StimuSplitter). Contractions were monitored by computer (Apple Macintosh LC) using a data recording and analysis system (MacLab) that was linked via preamplifiers (Macbridge) to Dynamometer UF1 transducers (Pioden Controls). It was not possible to reverse the inhibitory effect of cannabinoids on the twitch response by washing them out of the organ bath. Consequently only one concentration-response curve was constructed per tissue. In control experiments, Tween 80 was added instead of a drug. The control dose of Tween 80 was the same as the dose added in combination with the highest dose of drug used.

Strips of myenteric plexus-longitudinal muscle were dissected from the small intestine of male albino Dunkin-Hartley guinea-pigs (330–502 g) using the method of Paton and Zar (1968). Contractions were evoked by single

bipolar rectangular pulses of 110% maximal voltage, 0.5 ms duration and 0.1 Hz frequency. Drugs were added only after the twitch amplitude had become constant. Agonists were added cumulatively at intervals of 15 min. Antagonists were administered 15 min before the first addition of an agonist. Once a drug had been added, tissues were incubated for several hours without replacing the fluid in the bath.

Vasa deferentia were obtained from albino MF1 mice weighing 33–52 g. Contractions were evoked with 500 ms trains (0.1 Hz repetition rate) of three pulses at 5 Hz (110% maximal voltage and 0.5 ms pulse duration). Each vas deferens was subjected to more than one period of stimulation. The first of these began after the tissue had equilibrated but before drug administration and continued for 11 min. The stimulator was then switched off for a 10 min period after which tissues were subjected to further periods of stimulation, each lasting for 5 min. Baths were washed out by overflow at the end of each stimulation period. Drug additions were made immediately after the 11 min stimulation period (time zero) and also after each bath wash. The duration of the stimulation-free period that followed each addition of an agonist was 10 min. When antagonists were used, these were added at time zero, the first addition of agonist then being made after the bath wash at the end of the first 5 min stimulation period (+20 min).

2.4. In vivo experiments

Mice were subjected to a drug treatment shown previously to render the vasa deferentia of these animals cannabinoid tolerant (Pertwee et al., 1993). The treatment consisted of two intraperitoneal injections of Δ^9-tetrahydrocannabinol, given 24 h apart at a dose of 20 mg/kg. Animals were killed 24 h after the second injection and their vasa deferentia removed. Control animals received intraperitoneal injections of 40 mg/kg Tween 80. The injection volume was 0.25 ml/25 g.

2.5. Analysis of data

Values are expressed as means and limits of error as standard errors. Inhibition of the electrically evoked twitch response is expressed in percentage terms and has been calculated by comparing the amplitude of the twitch response after each addition of an agonist with its amplitude immediately before the first addition of the agonist. In experiments with the myenteric plexus-longitudinal muscle preparation, dose-response curves of WIN 55,212-2 were constructed in the presence of more than one concentration of O-823 and the dissociation constant (K_d) for the interaction between O-823 and cannabinoid receptors calculated from the slope ($1/K_d$) of the best-fit straight line of a plot of ($x - 1$) against B, constrained to pass through the origin (Tallarida et al., 1979). The equation for this graph is $(x - 1) = B/K_d$, where x (the 'dose ratio') is the dose of WIN 55,212-2 that produces a particular degree of inhibition in the presence of O-823 at a concentration, B, divided by the dose of WIN 55,212-2 that produces an identical degree of inhibition in the absence of O-823. Other K_d values were each calculated by substituting a single dose ratio value into the above equation. Dose ratio and potency ratio values and their 95% confidence limits have been determined by symmetrical (2 + 2) dose parallel line assays (Colquhoun, 1971) using responses to pairs of agonist concentrations located on the steepest part of each log concentration-response curve. In none of these assays did pairs of log concentration-response curves show significant deviation from parallelism ($P > 0.05$). Values with their 95% confidence limits for agonist concentrations producing 50% of their maximum inhibitory effect on the twitch response (EC_{50} values) have been calculated by non-linear regression analysis using GraphPAD InPlot (GraphPAD Software, San Diego, CA, USA).

3. Results

3.1. Binding and myenteric plexus-longitudinal muscle preparation experiments

It was found that O-823 readily displaced [^{3}H]CP 55,940 from its binding sites ($K_i = 0.77 \pm 0.05$ nM, $n = 3$). In the myenteric plexus-longitudinal muscle preparation, O-823 behaved as a potent competitive, surmountable antagonist of WIN 55,212-2 and CP 55,940, producing dextral shifts in the log concentration-response curves of both agonists without decreasing the size of their maximal effects (Fig. 2 and Table 1). The experiments with WIN 55,212-2 also showed the size of the dextral shift in its log concentration-response curve to be dependent on the concentration

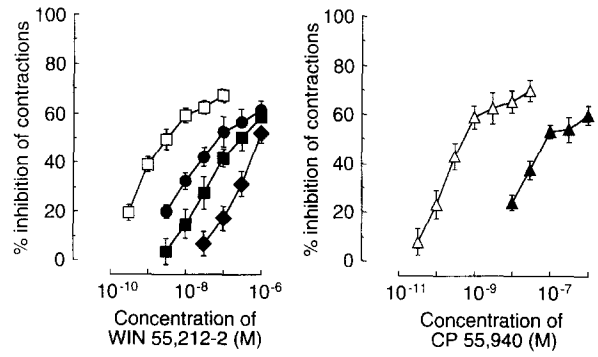

Fig. 2. Mean concentration-response curves for WIN 55,212-2 and CP 55,940 constructed in the presence of Tween 80 (open symbols) or of O-823 (filled symbols) at concentrations of 3.162 nM (circles), 31.62 nM (squares and triangles) or 316.2 nM (diamonds). Each symbol represents the mean value ± S.E. of inhibition of electrically evoked contractions of strips of myenteric plexus-longitudinal muscle expressed as a percentage of the amplitude of the twitch response measured immediately before the first addition of a twitch inhibitor to the organ bath ($n = 6$ or 8 different preparations).

Table 1
Dissociation constant (K_d) values of O-823 ($n = 6$ or 8)

Preparation	Agonist	K_d	
		Mean (nM)	95% confidence limits (nM)
Myenteric plexus	WIN 55,212-2	0.65 [a]	0.57 and 0.75 [a]
Myenteric plexus	CP 55,940	0.27	0.14 and 0.52
Vas deferens [b]	CP 55,940	0.26	0.17 and 0.39

[a] Calculated from experiments performed with Tween 80 and with O-823 at concentrations of 1, 3.162, 10, 31.62, 100, 316.2 and 1000 nM.
[b] Vasa deferentia were obtained from mice that had been pretreated with Δ^9-tetrahydrocannabinol.

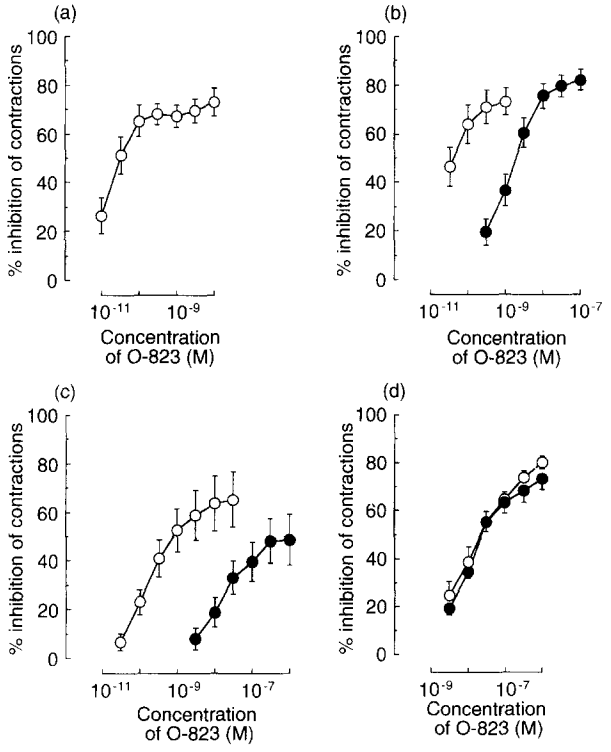

Fig. 3. Mean concentration-response curves for O-823 constructed (a) in the absence of other agents, (b) in the presence of 31.62 nM SR141716A (filled symbols) or of Tween 80 (open symbols), (c) in vasa deferentia obtained from mice pretreated with Δ^9-tetrahydrocannabinol (filled symbols) or Tween 80 (open symbols) and (d) in the presence of 31.62 nM SR141716A (filled symbols) or of Tween 80 (open symbols) using tissue obtained from mice pretreated with Δ^9-tetrahydrocannabinol. Each symbol represents the mean value ± S.E. of inhibition of electrically evoked contractions of vasa deferentia expressed as a percentage of the amplitude of the twitch response measured immediately before the first addition of O-823 to the organ bath ($n = 5$–12 different preparations). In (c), the ratio of the potency of O-823 in Tween 80 pretreated tissue to that in Δ^9-tetrahydrocannabinol pretreated tissue is 127.1, the 95% confidence limits being 52.3 and 333.0. These values were calculated by comparing the responses to 0.03162 and 0.3162 nM O-823 in Tween pretreated tissue with those to 3.162 and 31.62 nM O-823 in Δ^9-tetrahydrocannabinol pretreated tissue. The mean EC_{50} value of O-823 was 0.015 nM (95% confidence limits = 0.012 and 0.018 nM) in tissue obtained from untreated mice (a) and 0.19 nM (95% confidence limits = 0.14 and 0.26 nM) in tissue obtained from Tween 80 treated mice (c).

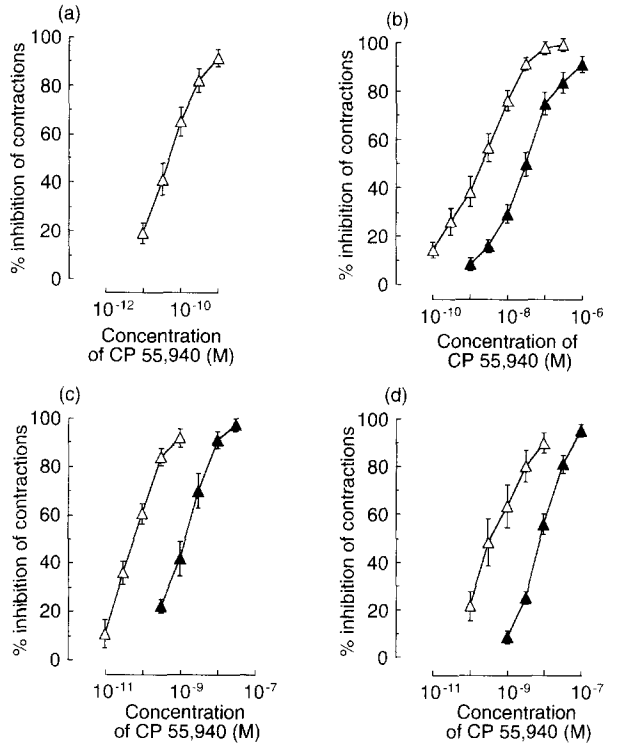

Fig. 4. Mean concentration-response curves for CP 55,940 constructed (a) in the absence of other agents, (b) in the presence of 3.162 nM O-823 (filled symbols) or of Tween 80 (open symbols) using vasa deferentia obtained from mice pretreated with Δ^9-tetrahydrocannabinol, (c) in the presence of 31.62 nM SR141716A (filled symbols) or of Tween 80 (open symbols) using tissue obtained from mice pretreated with Tween 80 and (d) in the presence of 31.62 nM SR141716A (filled symbols) or of Tween 80 (open symbols) using tissue obtained from mice pretreated with Δ^9-tetrahydrocannabinol. Each symbol represents the mean value ± S.E. of inhibition of electrically evoked contractions of vasa deferentia expressed as a percentage of the amplitude of the twitch response measured immediately before the first addition of CP 55,940 to the organ bath ($n = 6$–8 different preparations). The mean EC_{50} value of CP 55,940 was 0.05 nM (95% confidence limits = 0.04 and 0.06 nM) in tissue obtained from untreated mice (a) and 0.06 nM (95% confidence limits = 0.05 and 0.08 nM) in tissue obtained from Tween 80 treated mice (c).

of O-823 used (Fig. 2). By themselves, O-823 and Tween 80 did not significantly affect the amplitude of the twitch response.

3.2. Experiments with vasa deferentia obtained from unpretreated mice

O-823 shared the ability of the established cannabinoid receptor agonist, CP 55,940, to produce a concentration-related inhibition of electrically evoked contractions of the mouse vas deferens (Figs. 3 and 4). The mean EC_{50} value of O-823 was calculated to be 0.015 nM with 95% confidence limits 0.012 and 0.018 nM. CP 55,940 was approximately equipotent with O-823 but had a greater inhibitory effect at its maximal concentration. The effect of O-823 was susceptible to antagonism by 31.62 nM SR141716A (Fig. 3 and Table 2).

Table 2
Dissociation constant (K_d) values of SR141716A determined using the mouse isolated vas deferens ($n = 5–10$)

In vivo pretreatment	Agonist	K_d	
		Mean (nM)	95% confidence limits (nM)
None	O-823	0.71	0.18 and 1.65
Δ^9-Tetrahydrocannabinol	O-823	> 31.62 [a]	–
Tween 80	CP 55,940	1.42	0.89 and 2.34
Δ^9-Tetrahydrocannabinol	CP 55,940	1.73	1.08 and 2.94

Agonists were added in the presence of 31.62 nM SR141716A or Tween 80.
[a] No significant antagonism produced by 31.62 nM SR141716A.

3.3. Experiments with vasa deferentia obtained from mice pretreated with Δ^9-tetrahydrocannabinol or Tween 80

In vivo pretreatment of mice with Δ^9-tetrahydrocannabinol induced a dextral shift in the log concentration-response curve of O-823 in isolated vasa deferentia (Fig. 3). As a result an O-823 concentration of 3.162 nM, which markedly decreased the amplitude of electrically evoked contractions in tissues obtained from Tween 80 pretreated mice, had no inhibitory effect in Δ^9-tetrahydrocannabinol pretreated tissues. The same concentration of O-823 was found to antagonize CP 55,940 in Δ^9-tetrahydrocannabinol pretreated vasa deferentia, producing a clear dextral parallel shift in the log concentration-response curve of CP 55,940 (Fig. 4).

SR141716A was no less potent as an antagonist of CP55,940 in vasa deferentia obtained from Δ^9-tetrahydrocannabinol pretreated mice than in tissues obtained from Tween 80 pretreated animals (Fig. 4 and Table 2). However, SR141716A did not antagonize O-823 in vasa deferentia obtained from Δ^9-tetrahydrocannabinol pretreated mice (Fig. 3 and Table 2). By themselves, SR141716A and O-823 increased the amplitude of the twitch response in Δ^9-tetrahydrocannabinol or Tween 80 pretreated tissues whereas the addition of Tween 80 was followed by a small but statistically significant decrease in amplitude (Fig. 5).

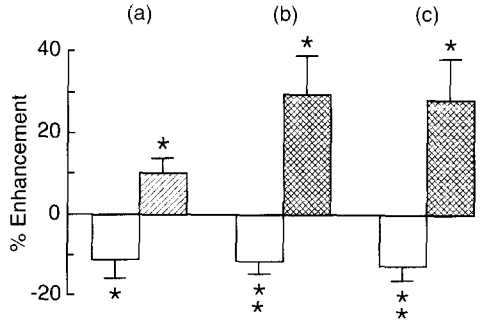

Fig. 5. Changes in the amplitudes of electrically evoked contractions following the administration to vasa deferentia of 3.162 nM O-823 (hatched columns), 31.62 nM SR141716A (cross-hatched columns) or Tween 80 (open columns). Preparations were exposed to these agents for 15 or 20 min. Tissues were obtained (a) and (b) from mice pretreated with Δ^9-tetrahydrocannabinol or (c) from mice pretreated with Tween 80. Each column represents the mean value ± S.E. of the change in the amplitude of contractions expressed as a percentage of the amplitude measured immediately before the addition of O-823, SR141716A or Tween 80 to the organ bath ($n = 8–12$ different preparations). For each column, the significance of the difference between the amplitude before and after addition of O-823, SR141716A or Tween 80 has been calculated by Student's t-test for paired data (* $P < 0.05$; ** $P < 0.01$).

4. Discussion

The results obtained from experiments with the myenteric plexus-longitudinal muscle preparation of guinea-pig small intestine showed O-823 to be an antagonist of CP 55,940 and WIN 55,212-2. There are several reasons for believing that O-823 may have antagonized these compounds by acting through cannabinoid CB_1 receptors. First, there is convincing evidence that the guinea-pig small intestine contains such receptors (Paterson and Pertwee, 1993; Pertwee et al., 1992, 1996). Second, the K_d values of O-823 determined in our experiments with the myenteric plexus-longitudinal muscle preparation are almost identical to its K_i value, calculated from the ability of O-823 to displace [^{3}H]CP 55,940 from cannabinoid binding sites in brain tissue. Finally, CP 55,940 and WIN 55,212-2 are both cannabinoid receptor agonists (Martin et al., 1995).

In contrast to its effect on the myenteric plexus-longitudinal muscle preparation, O-823 produced a marked inhibition of electrically evoked contractions of the mouse isolated vas deferens. This inhibitory effect was presumably mediated by cannabinoid CB_1 receptors since it could be prevented by the cannabinoid CB_1 receptor antagonist, SR141716A, and since the K_d value of SR141716A calculated from these data is similar to values obtained in previous vas deferens experiments with established cannabinoid receptor agonists (Pertwee et al., 1995). O-823 differs from cannabinoids such as Δ^9-tetrahydrocannabinol, WIN 55,212-2 and CP 55,940 (Pertwee et al., 1995) in that it does not inhibit the twitch response completely when administered at its maximal concentration. It is, therefore, a partial agonist. However, O-823 is no less potent than these other agents, producing 50% inhibition of electrically evoked contractions of mouse vasa deferentia at a concentration of 0.03 nM (Fig. 3). CP 55,940 was found to produce the same degree of inhibition at a concentration of 0.05 nM (Fig. 4) and previous experiments with vasa deferentia have shown Δ^9-tetrahydrocannabinol and WIN 55,212-2 to produce 50% inhibition of the twitch response at concentrations of 1.69 nM and 6.3 nM, respectively (Pertwee et al., 1992, 1995).

It has been found in previous experiments with the mouse vas deferens that the in vivo pretreatment with Δ^9-tetrahydrocannabinol used in the present investigation causes dextral shifts in the log concentration-response curves of CP 55,940, WIN 55,212-2 and anandamide but

no reduction in the size of the maximal effects of these agents (Pertwee et al., 1993). In line with these earlier findings, vasa deferentia taken from Δ^9-tetrahydrocannabinol pretreated mice showed significant tolerance to the inhibitory effects on the twitch response of both O-823 (Fig. 3) and CP 55,940 (Fig. 4). However, the dextral shift produced by this pretreatment in the log concentration-response curve of O-823 (127-fold) was considerably greater than that which has been observed previously in experiments with CP 55,940, WIN 55,212-2 or anandamide (8.7-, 9.6- and 12.3-fold, respectively) and was accompanied by a clear reduction in the size of the maximal response. One possible interpretation of these findings is that in vivo pretreatment of mice with Δ^9-tetrahydrocannabinol produces a decrease in cannabinoid receptor density. Such a decrease would be expected to have a more profound effect on the potency and the size of the maximal effect of O-823 than on those of CP 55,940, WIN 55,212-2 or anandamide since O-823 is a partial agonist whilst the other agents behave as full agonists in the mouse vas deferens (Pertwee et al., 1993, 1995). This is because, according to classical receptor theory, a partial agonist needs to occupy more receptors than a full agonist in order to produce an effect of any particular size. Indeed, it is generally considered that a partial agonist must occupy all its receptors to elicit its maximum response whereas a full agonist may need to occupy only a small proportion of the same receptor population to elicit its full response. The hypothesis that Δ^9-tetrahydrocannabinol pretreatment produces a decrease in cannabinoid receptor density within the vas deferens is supported by results from previous experiments (De Fonseca et al., 1994; Oviedo et al., 1993). These have shown that cannabinoid tolerance induced in rats by in vivo pretreatment with Δ^9-tetrahydrocannabinol or CP 55,940 is accompanied by significant reductions in the density of cannabinoid CB_1 binding sites in brain tissue but by little or no change in the affinity of these sites.

In vivo pretreatment of mice with Δ^9-tetrahydrocannabinol abolished the ability of a concentration of 3.162 nM of O-823 to inhibit electrically evoked contractions of the vas deferens, replacing it with an ability to antagonize CP 55,940-induced inhibition of such contractions. The high potency exhibited by O-823 as an antagonist in these experiments indicates that although the pretreatment with Δ^9-tetrahydrocannabinol may have reduced cannabinoid receptor density, it is unlikely to have produced much of a reduction in the affinity of cannabinoid receptors for O-823. Indeed, the K_d value of O-823 determined in experiments with CP 55,940 was found to be the same in Δ^9-tetrahydrocannabinol pretreated vasa deferentia as in the myenteric plexus-longitudinal muscle preparation, a finding which may well also indicate that O-823 blocks the same type of receptor in both tissues. The suggestion that the in vivo pretreatment with Δ^9-tetrahydrocannabinol used had little effect on cannabinoid receptor affinity is in line with results obtained in binding experiments with rat tissue (see above). It is also supported by the present finding that there is little difference between the K_d value of SR141716A obtained in CP 55,940 experiments with Δ^9-tetrahydrocannabinol pretreated vasa deferentia and that obtained in CP 55,940 experiments with vehicle pretreated tissue.

Although in vivo pretreatment with Δ^9-tetrahydrocannabinol did not affect the ability of SR141716A to antagonize CP 55,940 in the vas deferens, it did abolish the ability of this compound to antagonize O-823. This finding may be an indication that pretreatment with Δ^9-tetrahydrocannabinol depletes the vas deferens of cannabinoid CB_1 receptors to the extent that there are no longer enough of them to mediate responses to a partial agonist such as O-823 but still enough to mediate responses to a full agonist such as CP 55,940. A previous observation, that pretreatment with Δ^9-tetrahydrocannabinol reduces the maximal inhibitory effect as well as the potency of Δ^9-tetrahydrocannabinol itself in the mouse vas deferens (Pertwee et al., 1993), could also be explained in terms of a fall in cannabinoid receptor number. Further experiments are needed to investigate the effect of cannabinoid pretreatment on cannabinoid receptor density in the vas deferens and to identify the SR141716A-resistant mechanism by which O-823 induces its inhibitory effect in pretreated tissues. The effect of in vivo cannabinoid pretreatment on the affinity of cannabinoid receptors in the vas deferens also requires further investigation.

If a decrease in cannabinoid receptor density does cause O-823 to behave as an antagonist instead of a partial agonist, it is possible that O-823 may show agonist activity in the mouse vas deferens but behave as an antagonist in the myenteric plexus-longitudinal muscle preparation because there are fewer cannabinoid receptors in the second of these tissues than in the first. Consistent with this idea is the finding that maximal concentrations of cannabinoids such as Δ^9-tetrahydrocannabinol, CP 55,940 and WIN 55,212-2 abolish the twitch response completely in the vas deferens but produce only a partial inhibition in the myenteric plexus-longitudinal muscle preparation (Pertwee et al., 1992, 1993, 1995).

When administered by themselves, SR141716A and O-823 were found to produce increases in the amplitudes of electrically evoked contractions of Δ^9-tetrahydrocannabinol pretreated or unpretreated vasa deferentia. Similar results have been obtained previously in SR141716A experiments with the myenteric plexus-longitudinal muscle preparation (Pertwee et al., 1996). The mechanism(s) responsible for the stimulatory effects of SR141716A and O-823 on twitch amplitude have still to be determined. Also still to be explained is why O-823 (but not CP 55,940) was found to be about 12 times less potent as an inhibitor of the twitch response in vasa deferentia obtained from Tween 80 treated mice than in tissue from untreated animals (Figs. 3 and 4).

In conclusion, our results show O-823 to be a potent

partial cannabinoid receptor agonist. Further experiments are required to explore our hypothesis that this compound behaves as an antagonist in the myenteric plexus-longitudinal muscle preparation and in Δ^9-tetrahydrocannabinol pretreated vasa deferentia but as a partial agonist in unpretreated vasa deferentia because the first two of these preparations contain fewer cannabinoid receptors than the third. In view of the therapeutic potential of cannabinoids (Hollister, 1986; Pertwee, 1995) it will be important to establish the extent to which the combination of high potency and low efficacy shown by O-823 influences its benefit-to-risk ratio in the whole organism. We have already carried out preliminary in vivo experiments with O-823. These indicate that O-823 shares the ability of cannabinoid CB_1 receptor agonists to produce antinociception and to reduce spontaneous activity and body temperature in mice. Whether O-823 can also produce detectable antagonism in vivo remains to be established.

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