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(−)-Cannabidiol antagonizes cannabinoid receptor agonists and noradrenaline in the mouse vas deferens

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Abstract

The nonpsychoactive plant cannabinoid, (−)-cannabidiol, modulates in vivo responses to Δ^9-tetrahydrocannabinol. We have found that cannabidiol can also interact with cannabinoid CB_1 receptor agonists in the mouse vas deferens, a tissue in which prejunctional cannabinoid CB_1 receptors mediate inhibition of electrically evoked contractions by suppressing noradrenaline and/or ATP release. Cannabidiol (0.316–10 μM) attenuated the ability of (*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo-[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone (*R*-(+)-WIN55212) to inhibit contractions in a concentration-related, surmountable manner with a K_B value (120.3 nM) well below its reported cannabinoid receptor CB_1/CB_2 K_i values. Cannabidiol (10 μM) also antagonized (−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol (CP55940; $K_B = 34$ nM) and [D-Ala^2, NMePhe^4, Gly-ol]enkephalin (DAMGO; $K_B = 5.6$ μM) and attenuated contractile responses to noradrenaline, phenylephrine and methoxamine but not to β, γ-methyleneadenosine 5′-triphosphate. At 3.16–10 μM, it increased the amplitude of evoked contractions, probably by enhancing contractile neurotransmitter release. We conclude that cannabidiol antagonizes *R*-(+)-WIN55212 and CP55940 by acting at prejunctional sites that are unlikely to be cannabinoid CB_1 or CB_2 receptors.

Keywords: Cannabidiol; CP55940; WIN55212; Drug interaction; Cannabinoid CB_1 receptor; Vas deferens, mouse

1. Introduction

Cannabinoid CB_1 receptor agonists are already used clinically as anti-emetics and to stimulate appetite, and it is generally accepted that both these drugs and cannabis may have other therapeutic applications that include the relief of chronic neuropathic or inflammatory pain and the amelioration of spasticity caused by multiple sclerosis (see Pertwee, 2001, 2002; Pertwee and Ross, 2002). Cannabinoid CB_1 receptor agonists also produce some unwanted effects, for example, an impairment of short-term memory (Hampson and Deadwyler, 1999), prompting the need for strategies that will selectively reduce the incidence or intensity of these effects. There is evidence that one such strategy may be to administer the nonpsychotropic plant cannabinoid, (−)-cannabidiol (see Pertwee, 1988), together with a cannabinoid CB_1 receptor agonist. Thus, experiments with human subjects have indicated that doses of cannabidiol having no detectable effect by themselves can attenuate some of the unwanted effects of Δ^9-tetrahydrocannabinol, the main psychoactive constituent of cannabis (Karniol et al., 1974). These unwanted effects are Δ^9-tetrahydrocannabinol-induced anxiety, tachycardia and impairment of time sense. There is also evidence, albeit from experiments with mice, that cannabidiol has no effect on antinociception induced by oral Δ^9-tetrahydrocannabinol in the abdominal stretch test (Sanders et al., 1979) or, indeed, that it can potentiate the antinociceptive effect of intraperitoneal Δ^9-tetrahydrocannabinol in the hot plate test (Karniol and Carlini, 1973). In other experiments, however, cannabidiol has been found to attenuate antinociception induced by Δ^9-tetrahydrocannabinol in the mouse abdominal stretch test (Welburn et al., 1976). The mechanisms underlying these interactions between Δ^9-tetrahydrocannabinol and cannabidiol remain to be established. They are unlikely to involve direct interactions at cannabinoid receptor CB_1 and CB_2 binding sites except at rather high doses of cannabidiol. Thus, cannabidiol appears to have relatively low affinity for cannabinoid receptors: K_i values for cannabidiol-induced displacement of a radiolabelled ligand from cannabinoid

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CB_1 and CB_2 receptor binding sites have been reported to be 4.35 and 2.86 μM, respectively in one study (Showalter et al., 1996) and >10 μM in other experiments (Bisogno et al., 2001). It is important that interactions between cannabidiol and cannabinoid CB_1 or CB_2 receptor agonists are investigated not only as this may facilitate exploitation of the therapeutic potential of cannabis or of individual cannabinoids, but also because cannabis is used so extensively for recreational purposes and because the therapeutic potential of Δ^9-tetrahydrocannabinol in combination with cannabidiol is now being studied in the clinic (Whittle et al., 2001).

The first part of the present investigation was directed at exploring the ability of cannabidiol to modulate the inhibitory effect of the established cannabinoid CB_1 receptor agonist, (*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo-[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone (*R*-(+)-WIN55212; see Pertwee, 1999), on electrically evoked contractions of the mouse isolated vas deferens. This is a tissue in which prejunctional cannabinoid CB_1 receptors are thought to mediate inhibition of evoked release of contractile neurotransmitters (Trendelenburg et al., 2000; see also Pertwee, 1997; Schlicker and Kathman, 2001). These neurotransmitters are ATP, acting on postsynaptic P_{2x} purinoceptors, and noradrenaline, acting mainly on postsynaptic α_1-adrenoceptors (von Kügelgen and Starke, 1991). Our initial experiments showed that cannabidiol readily antagonizes *R*-(+)-WIN55212 and we therefore carried out further experiments to establish whether this antagonism takes place at prejunctional or postjunctional sites. We also went on to investigate the ability of cannabidiol to antagonize two other inhibitors of electrically evoked contractions of the mouse vas deferens. These were the established cannabinoid CB_1 receptor agonist, (−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol (CP55940; see Pertwee, 1999), and the μ-opioid receptor agonist, [D-Ala^2, NMePhe^4, Gly-ol]enkephalin (DAMGO) (Corbett et al., 1993; Pertwee and Griffin, 1995). Some of the results described in this paper have been presented to the British Pharmacological Society (Craib et al., 2002).

2. Materials and methods

2.1. Materials

Methoxamine hydrochloride, β, γ-methyleneadenosine 5′-triphosphate (β, γ-methylene-ATP), noradrenaline bitartrate, phenylephrine hydrochloride and prazosin hydrochloride were purchased from Sigma-Aldrich (Poole, Dorset, England), whereas *R*-(+)-WIN55212 mesylate, DAMGO and the tetrasodium salt of pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS) were obtained from Tocris (Bristol, England). CP55940 was supplied by Pfizer (Groton, CT), while samples of cannabidiol were obtained from Professor R. Mechoulam, Department of Natural Products, Hebrew University, Jerusalem and from the National Institute on Drug Abuse. Noradrenaline was dissolved in a 0.9% aqueous solution of NaCl (saline) containing ascorbic acid (100 μM). Cannabidiol, CP55940 and prazosin were dissolved in dimethyl sulphoxide (DMSO), and *R*-(+)-WIN55212 was dissolved in a 50% aqueous solution of DMSO (v/v). Other drugs were dissolved in saline. Drugs were added in a volume of 10 μl.

2.2. Experimental procedure

Vasa deferentia were obtained from albino MF1 mice weighing 30 to 50 g. Each tissue was mounted vertically in a 4-ml organ bath at an initial tension of 0.5 g. The baths contained Mg^{2+}-free Krebs solution which was kept at 35 to 36 °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. Concentration–contractile response curves of noradrenaline, methoxamine and β, γ-methylene-ATP were constructed cumulatively without washout, 30 min after the addition of cannabidiol or DMSO. Experiments with other drugs were performed with electrically stimulated vasa deferentia as described previously (Ross et al., 2001). Stimuli were generated by a Grass S48 stimulator, then amplified (channel attenuator; MedLab Instruments) and divided to yield separate outputs to four organ baths (StimuSplitter; MedLab Instruments). They were applied through a platinum electrode attached to the upper end and a stainless steel electrode attached to the lower end of each bath. Isometric contractions were recorded. These were monitored by computer (Apple Macintosh) using a data recording and analysis system (MacLab) that was linked via preamplifiers to Pioden UF1 (Harvard Apparatus) or MLT1030 transducers (ADInstruments). Unless stated otherwise, monophasic contractions were evoked by applying 0.5 s trains of pulses of 110% maximal voltage (train frequency 0.1 Hz; pulse frequency 5 Hz; pulse duration 0.5 ms).

After placement in an organ bath, each tissue was subjected to electrical stimulation consisting of a series of stepwise increases in voltage that progressed over a 10-min period from submaximal to supramaximal (110% maximal). Tissues then underwent an equilibration procedure in which they were subjected to alternate periods of stimulation (2 min) and rest (10 min) until consistent twitch amplitudes were obtained. In most experiments, an addition of cannabidiol, prazosin or DMSO was now made. In experiments with noradrenaline, methoxamine and phenylephrine, no further electrical stimuli were applied. In all other experiments, tissues were rested from stimulation for 28 min. They were then stimulated for 2 min before being subjected to the first of a series of additions of a twitch inhibitor or of PPADS or prazosin. Before the next addition, the tissues were rested for 3 or 28 min and then stimulated for 2 min. This cycle of drug addition, 3- or 28-min rest and 2-min

stimulation was then repeated to allow the construction of a concentration–response curve. For all experiments, only one concentration–response curve was constructed per tissue (Pertwee et al., 1996) and each of these was constructed cumulatively without washout. The method we used complies with the European Community guidelines for the use of experimental animals.

2.3. Analysis of data

Values have been expressed as means and variability as S.E.M. or as 95% confidence limits. For some experiments, the amplitudes of electrically evoked contractions have been expressed in grams, while drug-induced increases of electrically evoked contractions and contractile responses to drugs have been expressed as the increase in tension (g). Inhibition of the electrically evoked twitch response has been expressed in percentage terms and this has been calculated by comparing the amplitude of the twitch response after each addition of a twitch inhibitor with its amplitude immediately before the first addition of the inhibitor. Mean values have been compared using Student's two-tailed *t*-test for paired or unpaired data, one-way analysis of variance (ANOVA) followed by Dunnett's test (GraphPad Prism; GraphPad Software, San Diego, CA). A P value <0.05 was considered to be significant. Values for EC_{50}, for maximal effects (E_{max}) and for the S.E.M. or 95% confidence limits of these values have been calculated by nonlinear regression analysis using the equation for a sigmoid concentration–response curve (GraphPad Prism). Dose–response curves of *R*-(+)-WIN55212 were constructed in the presence of more than one concentration of cannabidiol, and the dissociation constant (K_B) of cannabidiol has been calculated from the slope ($1/K_B$) of the best-fit straight line of a plot of $(x-1)$ against B, constructed by linear regression analysis and constrained to pass through the origin (GraphPad Prism) (Tallarida et al., 1979). The equation for this graph is $(x-1)=B/K_B$, where x (the 'concentration ratio') is the concentration of a twitch inhibitor that produces a particular degree of inhibition in the presence of a competitive reversible antagonist at a concentration, B, divided by the concentration of the same twitch inhibitor that produces an identical degree of inhibition in the absence of the antagonist. K_B values of cannabidiol have also been calculated by substituting a single concentration ratio value into the above equation. The Schild slope for the interaction between *R*-(+)-WIN55212 and cannabidiol has been obtained from the best-fit straight line of a plot of log $(x-1)$ against log B (GraphPad Prism). The equation for this graph, $\log(x-1)=\log B-\log K_B$, predicts a slope of unity for all receptor-mediated interactions between agonists and antagonists that are competitive and reversible (Tallarida et al., 1979). Values of the concentration ratio and its 95% confidence limits were 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. This method was also used to establish whether two-point log concentration–response plots deviated significantly from parallelism.

3. Results

3.1. Effect of cannabidiol on the ability of R-(+)-WIN55212 to inhibit electrically evoked contractions of the vas deferens

Concentrations of cannabidiol ranging from 0.316 to 10 μM produced significant dextral shifts in the log concentration–response curve of *R*-(+)-WIN55212 for inhibition of electrically evoked contractions (Fig. 1). None of these dextral shifts deviated significantly from parallelism. Nor were they accompanied by any significant change in the maximal effect (E_{max}) of *R*-(+)-WIN55212 ($P>0.05$; ANOVA followed by Dunnett's test). The dextral shifts yielded a Schild plot with a slope that did not deviate significantly from unity (Fig. 1). The mean K_B value of cannabidiol calculated from these data by the Tallarida method (see Experimental procedure) was found to be 120.3 nM with 95% confidence limits of 99.7 and 141 nM.

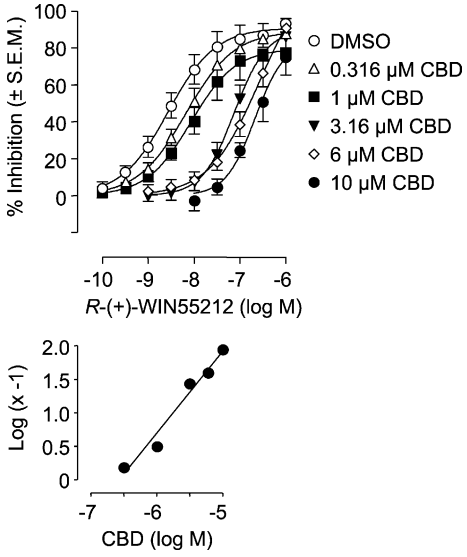

Fig. 1. Upper panel: The effect of pretreatment with cannabidiol (CBD) on the mean log concentration–response curve of *R*-(+)-WIN55212 in the mouse isolated vas deferens. Each symbol represents the mean value ± S.E.M. for inhibition of electrically evoked contractions expressed as a percentage of the amplitude of the twitch response measured immediately before the first addition of *R*-(+)-WIN55212 to the organ bath. Cannabidiol or DMSO was added 30 min before the first addition of *R*-(+)-WIN55212, further additions of which were made at 5-min intervals. Each log concentration–response curve was constructed cumulatively ($n=6$). Lower panel: Schild plot for antagonism of *R*-(+)-WIN55212 by 0.316–10 μM cannabidiol in which values for log $x-1$ were calculated from the data shown in the upper panel (x = concentration ratio). The slope of this plot with 95% confidence limits shown in parentheses is 1.2 (0.8 and 1.6).

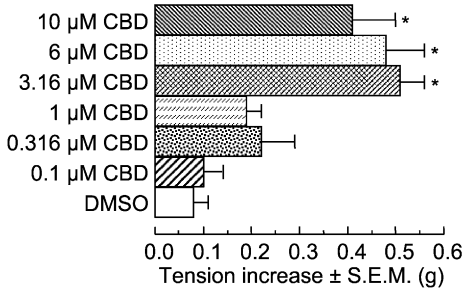

Fig. 2. Changes in the magnitude of electrically evoked contractions of vasa deferentia exposed for 30 min to cannabidiol (CBD) or DMSO. The symbols represent mean values ± S.E.M. of increases in tension. The asterisks indicate mean values that are significantly greater than those observed in the presence of DMSO ($P<0.0001$; ANOVA followed by Dunnett's test; $n=6$).

In the final 30-min period before the first addition of *R*-(+)-WIN55212, the amplitude of electrically evoked contractions did not increase significantly (paired *t*-test) when only DMSO was present (24.9 ± 8.2%; $P=0.06$; $n=6$). However, as shown in Fig. 2, mean twitch amplitude rose significantly above the control value in response to concentrations of cannabidiol above 1 μM. There was also a tendency for twitch amplitude to increase in the presence of lower cannabidiol concentrations. Because of this effect of cannabidiol, twitch amplitudes measured immediately before the first addition of *R*-(+)-WIN55212 (in order to calculate percentage inhibition of electrically evoked contractions; see Materials and methods) varied depending on the tissue pretreatment used. As a result, when twitch amplitude is expressed in grams, cannabidiol can be seen to have produced upward as well as rightward shifts in the log concentrations–response curve of *R*-(+)-WIN55212 (Fig. 3). Even so, EC_{50} values for *R*-(+)-WIN55212 calculated from the data shown in Fig. 3 were significantly increased by cannabidiol concentrations of 1 μM and above. Thus, for example, EC_{50} values with 95% confidence limits shown in brackets were 4.2 nM (1.8 and 9.9 nM) after DMSO and 27.9 nM (11.0 and 70.9 nM) after 1.0 μM cannabidiol ($n=6$).

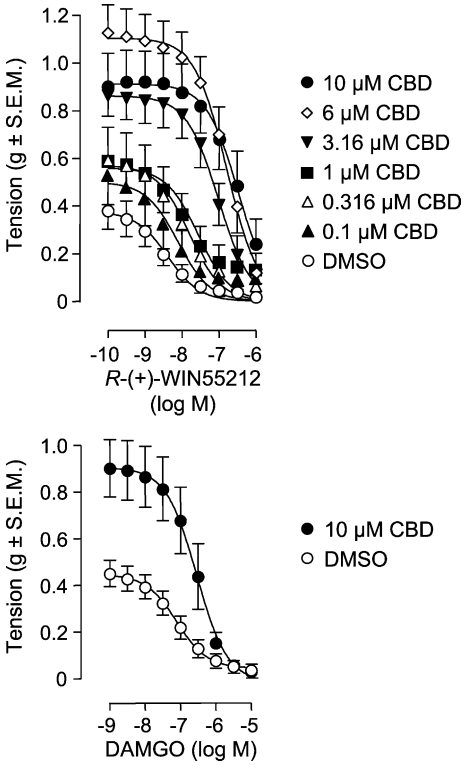

Fig. 3. The effect of pretreatment with cannabidiol (CBD) on the mean log concentration–response curve of *R*-(+)-WIN55212 in the mouse isolated vas deferens. The symbols represent mean values ± S.E.M. of the amplitude of the twitch response. Cannabidiol or DMSO was added 30 min before the first addition of *R*-(+)-WIN55212, further additions of which were made at 5-min intervals. Each log concentration–response curve was constructed cumulatively ($n=6$).

PPADS, a selective P_2 receptor antagonist (Ralevic and Burnstock, 1998), and prazosin, a selective α_1-adrenoceptor antagonist (Bylund et al., 1994), each produced a concentration-related inhibition of electrically evoked contractions in tissues that had been pretreated with 10 μM cannabidiol (Fig. 4A). The mean E_{max} of PPADS was 0.070 ± 0.004 g ($n=8$) and this is significantly less than the mean twitch amplitude in the presence of 100 nM prazosin (0.552 ± 0.019 g; $n=12$) ($P<0.001$; unpaired *t*-test). The corresponding values in the presence of DMSO (Fig. 4B) were 0.071 ± 0.009 g ($n=8$) and 0.330 ± 0.015 g ($n=12$), respectively, and these also differ significantly ($P<0.001$; unpaired *t*-test). The highest concentration of prazosin used markedly attenuated the contractile response of the vas deferens to noradrenaline. Thus, the mean contractile response to 10 μM noradrenaline was significantly less in

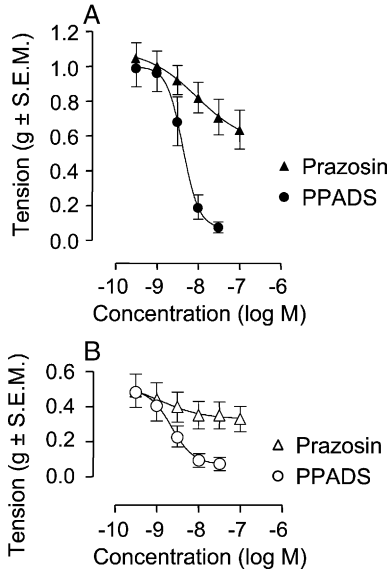

Fig. 4. The effect of PPADS ($n=8$) and prazosin ($n=12$) on the amplitudes of twitch responses of mouse isolated vasa deferentia following 30 min exposure to (A) 10 μM cannabidiol and (B) DMSO. Additions of PPADS and prazosin were made at 30-min intervals. The symbols represent mean values ± S.E.M. of the amplitude of the twitch response.

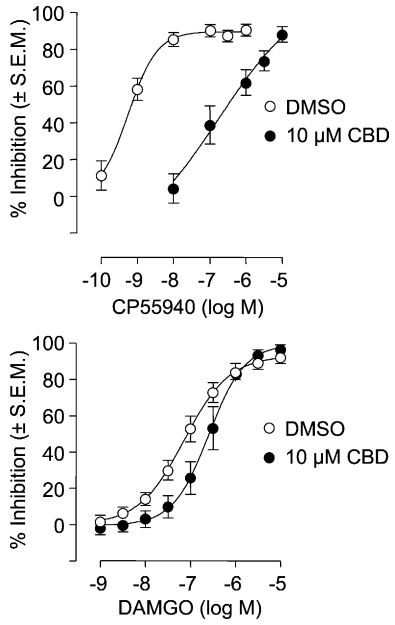

Fig. 5. The effect of pretreatment with 10 μM cannabidiol (CBD) on the mean log concentration–response curves of CP55940 ($n=7$) and DAMGO ($n=6$) in the mouse isolated vas deferens. Each symbol represents the mean value ± S.E.M. for inhibition of electrically evoked contractions expressed as a percentage of the amplitude of the twitch response measured immediately before the first addition of CP55940 or DAMGO to the organ bath. Cannabidiol or DMSO was added 30 min before the first addition of CP55940 or DAMGO, further additions of which were made at 30-min and 5-min intervals, respectively. Each log concentration–response curve was constructed cumulatively.

the presence of 100 nM prazosin (0.029 ± 0.037 g; $n=8$) than in the presence of DMSO (0.553 ± 0.076 g; $n=6$) ($P<0.001$; unpaired t-test).

3.2. Effect of cannabidiol on the ability of CP55940 and DAMGO to inhibit electrically evoked contractions of the vas deferens

Cannabidiol (10 μM) produced significant dextral shifts in the log concentration–response curves of CP55940 and DAMGO (Fig. 5), and as in the experiments with *R*-(+)-WIN55212, these shifts did not deviate significantly from parallelism. The K_B values of 10 μM cannabidiol against the two cannabinoid receptor agonists, CP55940 and *R*-(+)-WIN55212, calculated by applying the Schild equation, did not differ significantly from each other. However, these values were both significantly less than the K_B value of 10 μM cannabidiol against the μ-opioid receptor agonist, DAMGO. Thus, the mean K_B values of 10 μM cannabidiol against CP55940, *R*-(+)-WIN55212 and DAMGO with 95% confidence limits shown in parentheses were, respectively, 34 nM (11.7 and 90.2 nM), 114.1 nM (63.5 and 209.2 nM) and 5556 nM (3125 and 12500 nM). Like the E_{max} value of *R*-(+)-WIN55212 (see above), the E_{max} values of CP55940 and DAMGO were not reduced by cannabidiol. Thus, the mean E_{max} of CP55940 was 89.9 ± 3.0% in the presence of DMSO and this was not significantly greater than the mean inhibitory effect of 10 μM CP55940 in the presence of 10 μM cannabidiol (88.3 ± 4.2%) ($P=0.75$; unpaired t-test; $n=7$). E_{max} values of DAMGO in the presence of DMSO and 10 μM cannabidiol were 93.2 ± 3.9% and 99.5 ± 5.8%, respectively, and these values too are not significantly different ($P=0.39$; unpaired t-test; $n=6$).

EC_{50} values for CP55940 calculated from log concentrations–response curves in which the response was expressed in g tension were significantly less in DMSO pretreated tissues than in tissues that had been pretreated with 10 μM cannabidiol. These EC_{50} values with 95% confidence limits in parentheses are 0.53 nM (0.1 and 3.0 nM) and 95.2 nM (20.7 and 438.7 nM), respectively ($n=7$). The corresponding values for DAMGO, 77.6 nM (34.3 and 175.5 nM) and 287.4

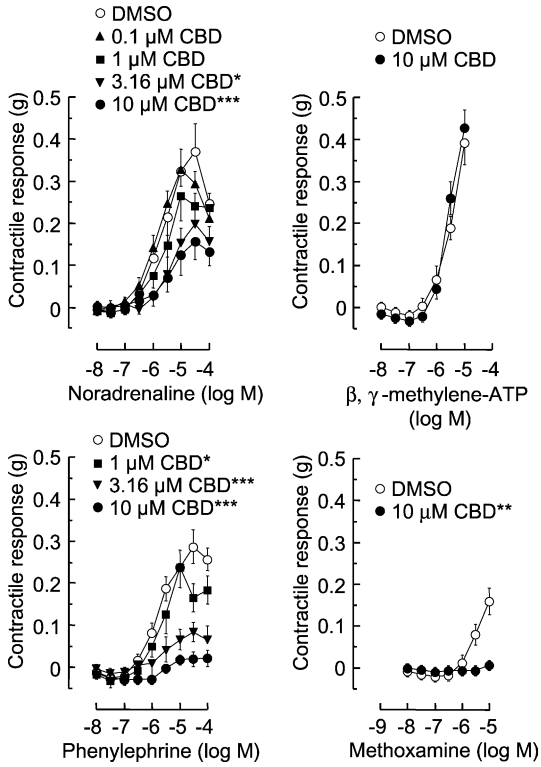

Fig. 6. The effect of pretreatment with cannabidiol (CBD) on the mean log concentration–response curves of noradrenaline ($n=8$ or 9), β, γ-methylene-ATP ($n=8$), phenylephrine ($n=6$ to 8) and methoxamine ($n=7$ or 8) in the mouse isolated vas deferens. Each symbol represents the mean tension increase ± S.E.M. Cannabidiol or DMSO was added 30 min before the first addition of contractile agent, each subsequent addition of which was made immediately after the effect of the previous addition had reached a plateau (dose cycles of 1 to 2 min). Log concentration–response curves were constructed cumulatively without washout and only one of these was constructed per tissue. The asterisks indicate that the mean tension increase in response to 31.6 μM noradrenaline or to 31.6 μM phenylephrine was significantly greater in the presence of DMSO than in the presence of cannabidiol (ANOVA followed by Dunnett's test), or that the mean tension increase in response to 10 μM methoxamine was significantly greater in the presence of DMSO than in the presence of 10 μM cannabidiol (unpaired t-test) (*$P<0.05$; **$P<0.01$; ***$P<0.001$).

nM (112.8 and 731.9 nM), respectively ($n = 6$), are not significantly different (Fig. 3).

3.3. Effect of cannabidiol on the ability of noradrenaline, methoxamine, phenylephrine and β, γ-methylene-ATP to induce contractions of the vas deferens

As shown in Fig. 6, cannabidiol markedly attenuated the contractile response of the vas deferens to noradrenaline, producing significant concentration-related reductions in the size of the maximal response to this adrenoceptor agonist. At 10 μM, cannabidiol also attenuated the contractile response of the vas deferens to the selective α_1-adrenoceptor agonists, phenylephrine and methoxamine (Fig. 6). However, this concentration of cannabidiol did not alter the sensitivity of vasa deferentia to the contractile effect of β, γ-methylene-ATP (Fig. 6). Because of the interactions that we had observed between cannabidiol and noradrenaline, phenylephrine and methoxamine, we also investigated whether the α_1-adrenoceptor antagonist, prazosin, shared the ability of cannabidiol to antagonize *R*-(+)-WIN55212. However, prazosin (100 nM) had no effect on the position of the log concentration–response curve of *R*-(+)-WIN55212 for inhibition of evoked contractions ($n = 5$; data not shown).

4. Discussion

Our initial experiments indicated that cannabidiol can attenuate the ability of *R*-(+)-WIN55212 to inhibit electrically evoked contractions of the mouse isolated vas deferens in a concentration-related manner. The data we obtained also suggest that cannabidiol did not produce this effect by acting postjunctionally to enhance smooth muscle sensitivity to ATP and noradrenaline, the main contractile transmitters of the mouse vas deferens. Thus, a concentration of cannabidiol (10 μM) that was markedly effective against *R*-(+)-WIN55212 did not alter the contractile potency of β, γ-methylene-ATP or increase the abilities of noradrenaline, phenylephrine and methoxamine to induce contractions. Indeed, 10 μM cannabidiol greatly reduced contractile responses of the vas deferens to noradrenaline, phenylephrine and methoxamine. It seems unlikely, therefore, that cannabidiol attenuated the inhibitory effect of *R*-(+)-WIN55212 by acting postjunctionally. One possibility is that cannabidiol acted at a prejunctional site to enhance the release of ATP and/or noradrenaline, thereby opposing the ability of *R*-(+)-WIN55212 to inhibit the evoked release of either or both of these contractile transmitters (Schlicker and Kathman, 2001; Trendelenburg et al., 2000; Pertwee, 1997). This hypothesis is supported by our findings that cannabidiol increased the amplitude of evoked contractions and yet did not enhance the contractile effects of β, γ-methylene-ATP or noradrenaline and also by a previous report that, at 10 and 100 μM, cannabidiol augments spontaneous release of noradrenaline and dopamine from rat brain synaptosomes (Poddar and Dewey, 1980). It is noteworthy that although we found cannabidiol to be effective against *R*-(+)-WIN55212 at concentrations of 0.316 μM and above (Fig. 1), it produced significant increases in amplitude only at the higher concentrations of 3.16, 6 and 10 μM. There was, however, a tendency for twitch amplitude to increase in response to lower concentrations of cannabidiol (Figs. 2 and 3).

Our finding that cannabidiol attenuated the contractile response of the vas deferens to noradrenaline yet elevated the twitch response and opposed *R*-(+)-WIN55212-induced inhibition of electrically evoked contractions prompts the hypothesis that, when our tissues were being stimulated electrically, the amplitude of the resulting twitches was determined predominantly by released ATP rather than by released noradrenaline. In support of this hypothesis, we found that twitch amplitude, both in the presence and absence of cannabidiol, was essentially abolished by the P_2 receptor antagonist, PPADS, but only partially reduced by the α_1-adrenoceptor antagonist, prazosin. This hypothesis is also consistent with our finding that cannabidiol did not attenuate the contractile response to β, γ-methylene-ATP and with evidence from other laboratories that the height of the twitch induced by electrical stimulation of the mouse vas deferens is determined to a greater extent by released ATP acting on rapidly signalling P_{2X} receptors than by released noradrenaline which acts on slower signalling α_1-adrenoceptors and is probably more important as a determinant of twitch duration (von Kügelgen and Starke, 1991).

Application of Schild analysis to the data shown in Fig. 1 indicates that within the concentration range of 0.316–10 μM, cannabidiol behaved as a competitive surmountable antagonist of *R*-(+)-WIN55212. This is in line with both an earlier report that cannabidiol can bind to cannabinoid CB_1 (and CB_2) receptors (see Introduction) and a previous observation that cannabidiol produced a parallel dextral shift in the log concentration–response curve for CP55940-induced stimulation of [^{35}S]GTPγS binding to rat cerebellar membranes (Petitet et al., 1998). However, 10 μM cannabidiol decreased the potency of CP55940 in the [^{35}S]GTPγS binding experiments by no more than 10-fold, much less than the decreases in the potencies of CP55940 and *R*-(+)-WIN55212 it produced in the present investigation. Moreover, the K_B values of cannabidiol that we calculated for its antagonism of these two cannabinoids are well below the reported K_i values of cannabidiol for displacing ligands from cannabinoid CB_1 and CB_2 receptors (see Introduction). Clearly, further experiments are required to establish more conclusively whether or not cannabidiol attenuated responses to CP55940 and *R*-(+)-WIN55212 in the vas deferens by acting as a competitive cannabinoid receptor antagonist. One possibility, that our K_B values for cannabidiol were influenced by its ability to increase the amplitude of electrically evoked contractions

of the vas deferens in the absence of other drugs, is unlikely. Thus, the cannabinoid CB_1 receptor antagonist/inverse agonist, SR141716A, shares the ability of cannabidiol to produce such increases in amplitude (Pertwee et al., 1996). Yet application of the method described in this paper yielded a K_B value of SR141716A for antagonism of CP55940-induced inhibition of electrically evoked contractions of the mouse vas deferens (1.42 nM; Pertwee et al., 1996) close to published K_D values of SR141716A for the cannabinoid CB_1 receptor determined from binding experiments with [^{3}H]SR141716A (0.19 to 1.24 nM; see Pertwee, 1999). It is also worth noting that when twitch amplitude is expressed in absolute terms (g tension) instead of percentage terms, we found that cannabidiol-induced antagonism of *R*-(+)-WIN55212 was still apparent (Fig. 3).

Breivogel et al. (2001) have detected the presence of a new type of cannabinoid receptor in brain tissue of cannabinoid CB_1 receptor knockout mice, while Járai et al. (1999) have obtained convincing evidence for the presence of another type of novel receptor in rat and mouse mesenteric arteries at which anandamide behaves as an agonist and cannabidiol as an antagonist. However, the interaction between cannabidiol and *R*-(+)-WIN55212 that we observed in the vas deferens was probably not mediated by receptors of the type proposed by Breivogel et al. (2001), as these can be activated by *R*-(+)-WIN55212 but not by CP55940, while we found CP55940 to be no less susceptible than *R*-(+)-WIN55212 to antagonism by cannabidiol. The receptors proposed by Járai et al. (1999) can also be excluded as they are not activated by *R*-(+)-WIN55212. Hence, the question of how cannabidiol produces its various effects in the vas deferens remains to be resolved. Whatever its mode(s) of action, these must confer some degree of selectivity. Thus, firstly, cannabidiol attenuated contractions induced by noradrenaline, phenylephrine and methoxamine without affecting those induced by β, γ-methylene-ATP, and secondly, cannabidiol was markedly less effective against DAMGO than against *R*-(+)-WIN55212 or CP55940.

In summary, we have identified a novel bioassay for investigating the pharmacological actions of cannabidiol in vitro and for elucidating the underlying structure–activity relationships. By itself, cannabidiol increased the amplitude of electrically evoked contractions of the mouse vas deferens, most probably mainly by enhancing the evoked neuronal release of ATP. Cannabidiol opposed the ability of *R*-(+)-WIN55212, CP55940 and DAMGO to inhibit electrically evoked contractions of this tissue, apparently by acting on prejunctional sites that are unlikely to be cannabinoid CB_1 receptors. It was equally effective against the two cannabinoids, *R*-(+)-WIN55212 and CP55940, but much less effective against the μ-opioid receptor agonist, DAMGO, suggesting a selective mode of action. Further experiments are now required to establish both the detailed mechanism(s) underlying the effects of cannabidiol on the mouse vas deferens and whether similar effects can be induced by cannabidiol in other tissues or organs including the brain. The extent to which such effects contribute to the in vivo pharmacology of cannabidiol and to its ability to modulate some in vivo effects of Δ^9-tetrahydrocannabinol also merits further investigation, especially since mixtures of Δ^9-tetrahydrocannabinol and cannabidiol are now being investigated in clinical trials (Whittle et al., 2001).

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