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Evidence for the presence of cannabinoid CB_1 receptors in mouse urinary bladder

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1 CP 55,244, (−)-11-hydroxy-dimethylheptyl-Δ^8-tetrahydrocannabinol, WIN 55,212-2, Δ^9-tetrahydrocannabinol, nabilone and anandamide each inhibited electrically-evoked contractions of the mouse isolated urinary bladder in a concentration-related manner, their EC_{50} values being respectively 15.9, 18.27, 27.23, 1327.6, 1341.5 and 4950.3 nM. (+)-11-hydroxy-dimethylheptyl-Δ^8-tetrahydrocannabinol was inactive at the highest concentration used (10 μM).

2 SR141716A (31.62 or 100 nM) produced parallel rightward shifts in the log concentration-response curves of CP 55,244, (−)-11-hydroxy-dimethylheptyl-Δ^8-tetrahydrocannabinol, WIN 55,212-2, Δ^9-tetrahydrocannabinol and anandamide for inhibition of electrically-evoked bladder contractions. The effect of the antagonist on the log concentration-response curve of CP 55,244 was shown to depend on the concentration of SR141716A used (31.62 to 1000 nM).

3 The amplitudes of contractions evoked by acetylcholine or β, γ-methylene-L-ATP were not decreased by 316.2 nM CP 55,244 or 3162 nM Δ^9-tetrahydrocannabinol. Electrically-evoked contractions were almost completely abolished by 200 nM tetrodotoxin.

4 The above results support the hypothesis that mouse urinary bladder contains prejunctional CB_1 cannabinoid receptors which can mediate inhibition of electrically-evoked contractions, probably by reducing contractile transmitter release.

5 AM 630 which behaves as a cannabinoid receptor antagonist in the mouse isolated vas deferens did not antagonize the ability of CP 55,244 or Δ^9-tetrahydrocannabinol to inhibit electrically-evoked contractions of the mouse bladder.

6 SR141716A produced small but significant increases in the amplitude of electrically-evoked contractions of the bladder suggesting that this tissue may release an endogenous cannabinoid receptor agonist or that some cannabinoid receptors in this tissue are precoupled to the effector system.

Keywords: Bladder; cannabinoid receptor agonists; cannabinoid receptor antagonist; Δ^9-tetrahydrocannabinol; AM 630; SR141716A

Introduction

Previous experiments have shown that cannabinoids can inhibit electrically-evoked contractions of isolated tissue preparations including the vas deferens of mouse, rat and guinea-pig and myenteric plexus-longitudinal muscle preparations of mouse and guinea-pig small intestine (Pacheco *et al.*, 1991; Pertwee *et al.*, 1992; 1993). In this investigation we have looked to see whether the ability of cannabinoids to inhibit electrically-evoked contractions of innervated smooth muscle preparations extends to the mouse urinary bladder, there being evidence that cannabinoids can relieve bladder spasms in patients with multiple sclerosis (see Pertwee, 1995). Experiments were carried out with representatives of all known chemical classes of cannabinoid receptor agonist: Δ^9-tetrahydrocannabinol, CP 55,244, WIN 55,212-2 and anandamide (see Pertwee, 1995). The effect of nabilone, a synthetic analogue of Δ^9-tetrahydrocannabinol, was also investigated as this cannabinoid has been successfully administered to a multiple sclerosis patient (Martyn *et al.*, 1995). As the results obtained indicated that cannabinoids can inhibit electrically-evoked contractions of the mouse bladder, further experiments were carried out to establish whether this effect is mediated by cannabinoid receptors. This was achieved firstly by carrying out experiments with the selective cannabinoid CB_1 receptor antagonist, SR141716A (Rinaldi-Carmona *et al.*, 1994) and secondly by comparing the potencies of (+)-and (−)-11-hydroxy-1′, 1′-dimethyl-heptyl-delta-8-tetrahydrocannabinol, the stereochemical purity of which is particularly high (see Mechoulam & Fride, 1995). Experiments were also carried out with AM 630 (Pertwee *et al.*, 1995a) which, like SR141716A (Rinaldi-Carmona *et al.*, 1994; Pertwee *et al.*, 1995b), behaves as a competitive cannabinoid receptor antagonist in the mouse isolated vas deferens.

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Methods

Electrical stimulation

Urinary bladders were obtained from albino MF1 mice weighing 32 to 59 g. Each animal was killed by cervical dislocation and the whole bladder removed and placed in Krebs solution at 37°C. Two parallel longitudinal cuts were now made, one along each lateral wall so that the upper and lower walls could be drawn apart to form a single strip with the apex of the bladder at its midpoint. Each strip was mounted vertically in a 4 ml organ bath at an initial tension of 0.5 g. 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, $MgSO_4.7H_2O$ 1.29, KH_2PO_4 1.19, $NaHCO_3$ 25.0, glucose 11.0 and $CaCl_2.6H_2O$ 2.54. Isometric contractions were evoked by stimulation with 0.5 s trains of three pulses of 110% maximal voltage (train frequency 0.1 Hz; pulse duration 0.5 ms) through platinum and stainless steel electrodes attached to the upper and lower ends of each bath respectively. 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 Performa 475) by use of a data recording and analysis system (MacLab) that was linked via preamplifiers (Macbridge) to UF1 transducers (Morro Bay).

Tissues were subjected to several periods of stimulation, each followed by a stimulation-free period (see below). The first period of stimulation was initiated after the tissue had equilibrated and continued for 10 min. Subsequent stimulation periods lasted 5 min and after each of these, the bath was washed out by overflow.

Experiments without SR141716A or Tween 80 pretreatment

These experiments were performed with CP 55,244, (+)-and (−) - 11 - hydroxy - dimethylheptyl - Δ^8 - tetrahydrocannabinol, WIN 55,212-2, Δ^9-tetrahydrocannabinol, nabilone, anandamide and AM 630. Drug additions were made immediately after the 10 min stimulation period (time zero) and also after each bath wash. Each stimulation free period that followed a drug addition lasted 80 min for 11-hydroxy-dimethylheptyl-Δ^8-tetrahydrocannabinol and 50 min for the other compounds tested.

Experiments with SR141716A

SR141716A was added immediately after the first stimulation period of 10 min (time zero) after which there was a stimulation-free period of 25 min. Additions of SR141716A followed by agonist were made after each 5 min stimulation period and subsequent bath wash. The stimulation-free period after each addition of agonist again lasted 10 min for WIN 55,212-2, 70 min for 11-hydroxy-dimethylheptyl-Δ^8-tetrahydrocanna-binol and 40 min for the other compounds tested. Control experiments were carried out with the same doses of Tween 80 (0.12 to 3.7 μg) as those added in combination with SR141716A. These experiments indicated that Tween 80 had no significant effect on the position of the log concentration-response curve of any of the agonists under investigation (data not shown). 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.

Contractions induced by acetylcholine and β,γ-methylene-L-ATP

Non-cumulative concentration-response curves were constructed in the absence and then in the presence of 316.2 nM CP 55,244, 3162 nM Δ^9-tetrahydrocannabinol or Tween 80. Dose-cycles were 2 min for acetylcholine and 15 min for β, γ-methylene-L-ATP. Contractions were registered on a polygraph recorder (Grass model 7D) by use of Pye Ether UF1 transducers.

Drugs

The (+) and (−) enantiomers of 11-hydroxy-dimethylheptyl-Δ^8-tetrahydrocannabinol were synthesized by Professor Raphael Mechoulam, University of Jerusalem, and AM 630, 6-iodo-pravadoline and anandamide by Professor Alexandros Makriyannis, University of Connecticut. Δ^9-tetrahydrocannabinol was obtained from the National Institute on Drug Abuse, CP 55,244, 4-[4-(1,1-dimethyl-heptyl)-2-hydroxyphenyl]-6-hydroxymethyl-decahydronaphthalen-2-ol from Pfizer, nabilone from Lilly, WIN 55,212-2, mesylate (*R*)-(+)-[2,3-dihydro-5-methyl-3-[(4-morpholino)methyl]pyrrolo-[1,2,3-de]-1,4-benzoxazin-6-yl](1-naphthyl)methanone from Sanofi Winthrop and SR141716A, N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2, 4 - dichlorophenyl) - 4 - methyl - 1H - pyrazole - 3 - carboxamide hydrochloride from Sanofi. Acetylcholine chloride was supplied by Sigma and β, γ-methylene-L-ATP by Research Biochemicals International. These were both dissolved in saline. Tetrodotoxin (Sigma) was dissolved in Krebs solution. Other 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.

Analysis of data

Values are expressed as means and limits of error as standard errors. The degree of inhibition of the twitch response by cannabinoid receptor agonists is expressed in percentage terms. This was calculated by comparing the amplitude of the electrically-evoked twitch response immediately before agonist administration with the amplitude of the twitch response at various times after agonist administration. The concentration of a cannabinoid that produced 50% of its maximum inhibitory effect on the twitch response (EC_{50}) was used to characterize its potency. Contractile responses to acetylcholine and β, γ-methylene-L-ATP are expressed in g.

In experiments with SR141716A, concentration-response curves of CP 55,244 were constructed in the presence of several concentrations of the antagonist so that K_D values could be 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 'concentration-ratio') is the concentration of an agonist that produces a particular degree of inhibition in the presence of SR141716A at a concentration, B, divided by the concentration of the same agonist that produces an identical degree of inhibition in the absence of SR141716A.

Concentration-ratio values and their 95% confidence limits were determined by symmetrical (2+2) dose parallel line assays (Colquhoun, 1971), by use of responses to pairs of agonist concentrations located on the steepest part of each log concentration-response curve. K_D values of SR141716A determined from experiments with agonists other than CP 55,940 were each calculated by substituting a single concentration-ratio value into the above equation. Non-linear or linear regression analysis was used to calculate EC_{50} values, values of $1/K_D$ and 95% confidence limits (GraphPAD In-Plot, GraphPAD Software, San Diego). Mean values were compared by Student's *t* test for unpaired data or by analysis of variance followed by Scheffé's test (Super Anova, Abacus Concepts Inc., Berkeley). A P value < 0.05 was considered to be significant.

Results

Effects of cannabinoid receptor agonists on evoked bladder contractions

CP 55,244, (−)-11-hydroxy-dimethylheptyl-Δ^8-tetrahydrocannabinol, WIN 55,212-2, Δ^9-tetrahydrocannabinol and anandamide all inhibited electrically-evoked contractions of the mouse isolated bladder. Nabilone was also inhibitory, its log concentration-response curve being almost identical to that of Δ^9-tetrahydrocannabinol. (−)-11-hydroxy-dimethylheptyl-Δ^8-tetrahydrocannabinol took 75 min to produce its full inhibitory effects on electrically-evoked contractions whereas the other cannabinoids took 45 min (data not shown). The inhibitory effect of cannabinoids was found to be dose-related (Figure 1) and their log concentration-response curves to be essentially sigmoid in shape ($r^2 = \text{or} > 0.95$). Mean EC_{50} values and r^2 values calculated by non-linear regression analysis are given in Table 1. The electrically-evoked twitch response of the bladder was not affected by (+)-11-hydroxy-dimethylheptyl-Δ^8-tetrahydrocannabinol, even at a concentration of 10 μM (Figure 1).

The amplitudes of contractions of the bladder elicited by acetylcholine or β, γ-methylene-L-ATP were not decreased by a concentration of Δ^9-tetrahydrocannabinol that produced a marked inhibition of electrically-evoked contractions (Figures 1 and 2). CP 55,244 (316.2 nM) also had no inhibitory effect on contractile responses of the bladder to either acetylcholine or β, γ-methylene-L-ATP (data not shown). Tetrodotoxin (200 nM) depressed the contractile response of the bladder to electrical stimulation by 86.5 ± 1.8% ($n = 6$).

Effects of SR141716A and AM 630

As shown in Figures 3 and 4, concentrations of SR141716A ranging from 31.62 to 1000 nM produced rightward shifts in the log concentration-response curves of CP 55,244, (−)-11-hydroxy-dimethylheptyl-Δ^8-tetrahydrocannabinol, WIN 55,212-2, Δ^9-tetrahydrocannabinol, nabilone and anandamide. None of these rightward shifts deviated significantly from parallelism. The effect of SR141716A on the ability of CP 55,244 to inhibit electrically-evoked bladder contractions was found to be dose-related (Figure 4). K_D values of SR141716A calculated from our data by Schild analysis are listed in Table 1. These suggest that the cannabinoid receptor agonists used in the present investigation do not differ significantly in their susceptibility to antagonism by SR141716A. At concentrations of 100 nM or more SR141716A produced small but significant increases in the amplitude of electrically-evoked contractions of the bladder (Figure 5).

AM 630 (1000 nM) did not significantly alter the position of the log concentration-response curve of either Δ^9-tetrahydrocannabinol or CP 55,244 (data not shown). Nor did concentrations of AM 630 ranging from 100 to 3162 nM have any detectable effect on the amplitude of electrically-evoked contractions of the bladder.

Discussion

The results obtained show that a range of cannabinoid CB_1 receptor agonists have the ability to inhibit electrically-evoked contractions of the mouse isolated urinary bladder in a concentration-related manner. The inhibitory effects of all these agonists were attenuated by submicromolar concentrations of the selective cannabinoid receptor antagonist, SR141716A, (Rinaldi-Carmona *et al.*, 1994), pointing to an involvement of cannabinoid receptors. We also found that whereas (−)-11-hydroxy-dimethylheptyl-Δ^8-tetrahydrocannabinol is a potent inhibitor of electrically-evoked bladder contractions, the corresponding (+)-enantiomer has no inhibitory effect even at a concentration of 10 μM. Because (−)-11-hydroxy-di-

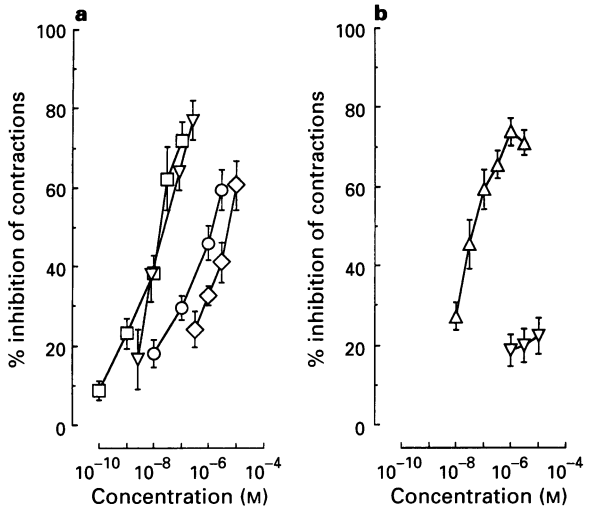

Figure 1 Mean concentration-response curves for (a) CP 55,244 (□), WIN 55,212-2 (▽), Δ^9-tetrahydrocannabinol (○) and anandamide (◇) and (b) (−)- and (+)-11-hydroxy-dimethylheptyl-Δ^8-tetrahydrocannabinol (△ and ▽, respectively). Each symbol represents the mean value of inhibition of electrically-evoked contractions expressed as a percentage of the amplitude of the twitch response measured immediately before the first addition of a compound to the organ bath ($n=5$ to 8 different bladders for each agonist); vertical lines indicate s.e.mean.

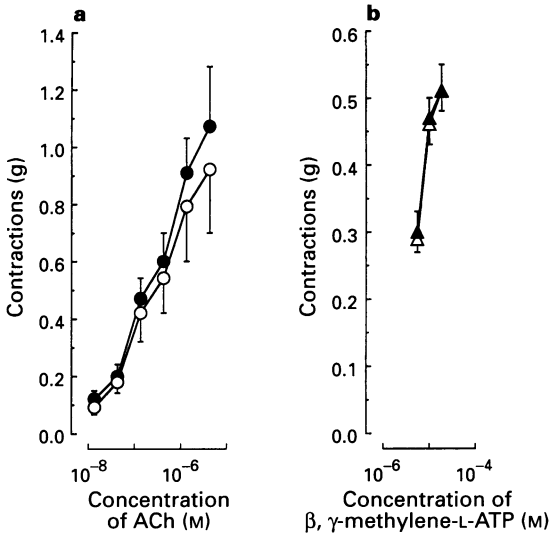

Figure 2 Mean concentration-response curves for (a) acetylcholine and (b) β, γ-methylene-L-ATP constructed in the presence of 3162 nM Δ^9-tetrahydrocannabinol (●, ▲) or Tween 80 (○, △). Each symbol represents the mean value of the amplitude of contractions produced by acetylcholine ($n=6$) or β, γ-methylene-L-ATP ($n=3$); vertical lines indicate s.e.mean. Δ^9-tetrahydrocannabinol and Tween 80 were added 45 min before the first addition of acetylcholine or β, γ-methylene-L-ATP.

Table 1 EC_{50} values of various cannabinoid receptor agonists and dissociation constant (K_D) values of SR141716A determined in the presence of these agonists

Cannabinoid	EC_{50} *Mean* (nM)	EC_{50} *95% confidence limits* (nM)	r^2	K_D *Mean* (nM)	K_D *95% confidence limits* (nM)
CP 55,244	15.90	8.18 & 30.90	0.978	7.62	6.85 & 8.58
WIN 55,212-2	27.23	11.47 & 64.64	0.982	8.88	4.70 & 16.86
AM 630	>3162	–	–	ND	–
HU 210	18.27	12.47 & 26.77	0.991	17.98	1.81 & 160.15
HU 211	>10,000	–	–	ND	–
THC	1327.6	561.8 & 3137.5	0.980	15.98	0.24 & 153.60
Nabilone	1341.5	522.0 & 3447.5	0.950	2.60	0.42 & 9.03
Anandamide	4950.3	2074.0 & 11814.1	0.967	3.40	0.72 & 7.21

HU 210 = (−)-11-hydroxy-1′,1′-dimethylheptyl-Δ^8-tetrahydrocannabinol; HU 211 = (+)-11-hydroxy-1′,1′-dimethylheptyl-Δ^8-tetrahydrocannabinol; THC = Δ^9-tetrahydrocannabinol.
K_D values were determined with SR141716A concentrations of 31.62, 100, 316.2 and 1000 nM (CP 55,244) or a single concentration of 31.62 nM (anandamide) or 100 nM (WIN 55,212-2, HU 210, THC and nabilone). ND = not determined.

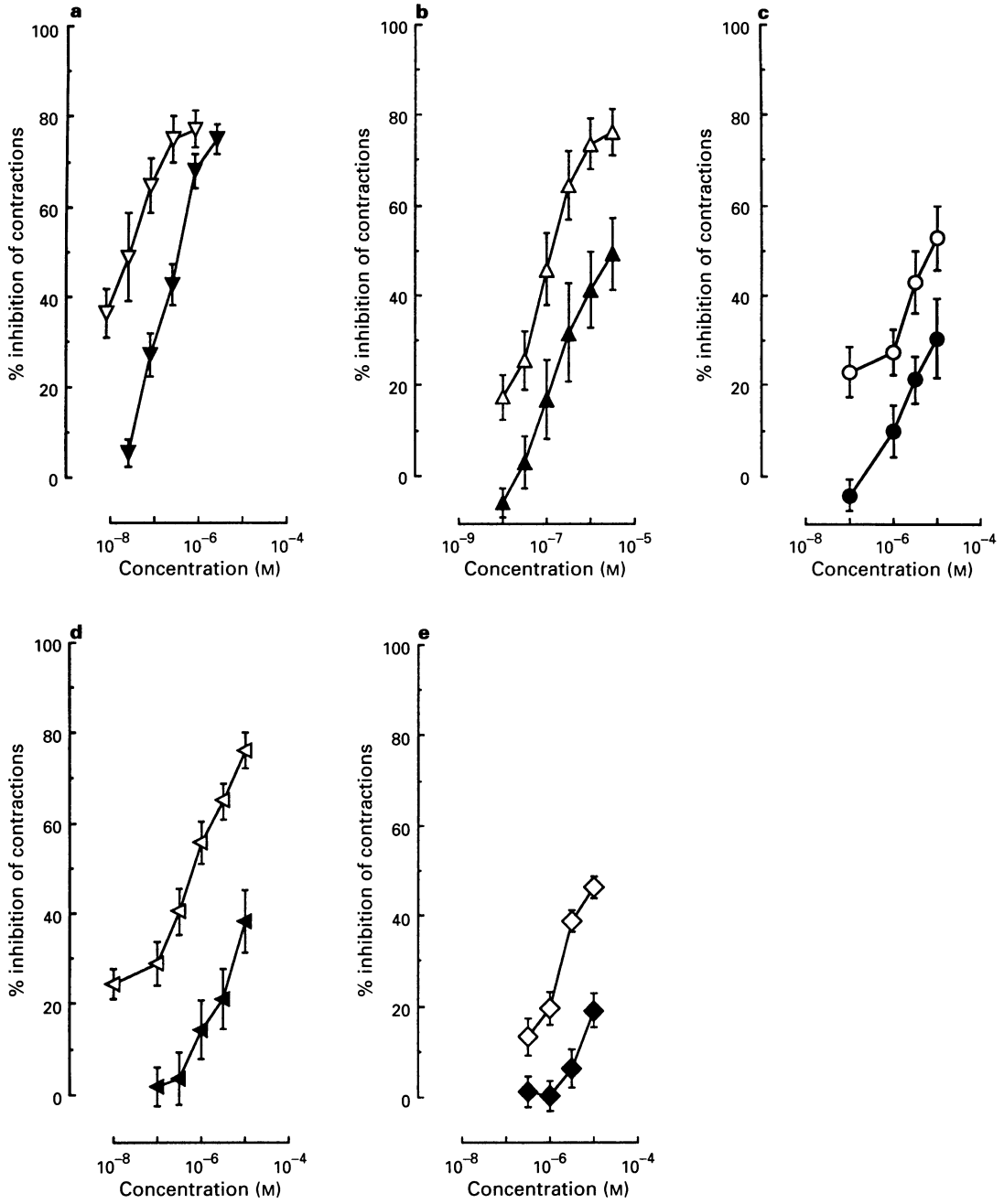

Figure 3 Mean concentration-response curves for (a) WIN 55,212-2, (b) (−)-11-hydroxy-dimethylheptyl-Δ^8-tetrahydrocannabinol, (c) Δ^9-tetrahydrocannabinol, (d) nabilone and (e) anandamide constructed in the presence of SR141716A (closed symbols) or Tween 80 (open symbols). The concentration of SR141716A was either 100 nM [(a) to (d)] or 31.62 nM (e). Each symbol represents the mean value of inhibition of electrically-evoked contractions expressed as a percentage of the amplitude of the twitch response measured immediately before the first addition of an agonist to the organ bath ($n = 5$ or 6 different bladders); vertical lines indicate s.e.mean. SR141716A and Tween 80 were added 30 min before the first addition of agonist.

methylheptyl-Δ^8-tetrahydrocannabinol has a far greater affinity for cannabinoid CB_1 and CB_2 binding sites than its (+)-enantiomer (Compton *et al.*, 1993; Slipetz *et al.*, 1995), this observation is a further indication that cannabinoid-induced inhibition of electrically-evoked bladder contractions may be mediated by cannabinoid receptors. There is some evidence to suggest that these receptors are CB_1 rather than CB_2 receptors. Thus the K_D values of SR141716A calculated from our data (Table 1) differ little from the K_i value of SR141716A determined from its ability to compete with [^{3}H]-CP 55,940 for CB_1 binding sites; the affinity of SR141716A for CB_2 binding sites has been found to be markedly less than its affinity for CB_1 binding sites (Rinaldi-Carmona *et al.*, 1994; Felder *et al.*, 1995).

The involvement of cannabinoid CB_2 receptors in the effects of cannabinoids on the bladder that we describe in this paper cannot yet be ruled out altogether. Thus for several of the cannabinoids that we used, there is good agreement between rank order of potency for inhibition of electrically-evoked bladder contractions and that for competition with a radiolabelled probe for CB_2 binding sites [(−)-11-hydroxy-dimethylheptyl-Δ^8-tetrahydrocannabinol > WIN 55,212-2 >> Δ^9-tetrahydrocannabinol > anandamide >> (+)-11-hydroxy-dimethylheptyl-Δ^8-tetrahydrocannabinol] (Felder *et al.*, 1995; Slipetz *et al.*, 1995). In addition, although WIN 55,212-2 is more potent than Δ^9-tetrahydrocannabinol both as an inhibitor of electrically-evoked bladder contractions and as a CB_2 receptor agonist, it is less potent than Δ^9-tetra-

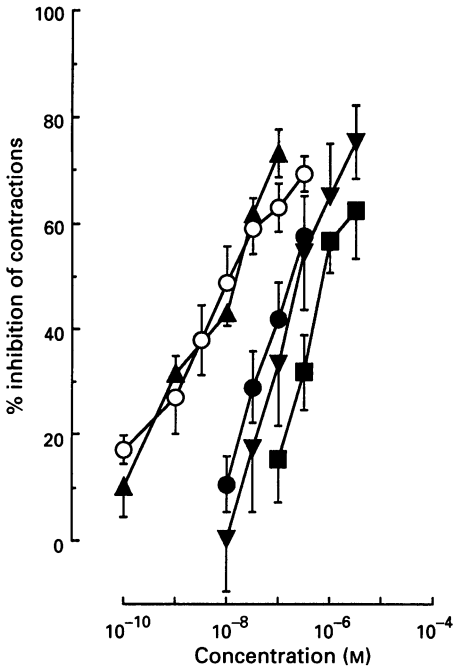

Figure 4 Mean concentration-response curves for CP 55,244 constructed in the presence of SR141716A at concentrations of 31.62 nM (▲), 100 nM (●), 316.2 nM (▼) or 1000 nM (■) or in the presence of Tween 80 (○). Each symbol represents the mean value of inhibition of electrically-evoked contractions expressed as a percentage of the amplitude of the twitch response measured immediately before the first addition of an agonist to the organ bath ($n=6$ or 8 different bladders); vertical lines indicate s.e.mean. SR141716A and Tween 80 were added 30 min before the first addition of CP 55,244.

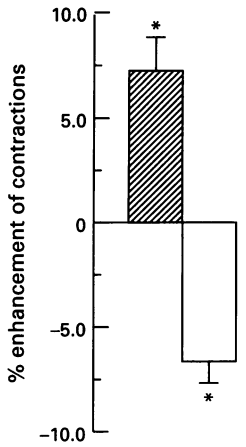

Figure 5 Effect of SR141716A on electrically-evoked contractions of strips of bladder observed just before the construction of the concentration-response curves shown in Figures 3 and 4. Preparations were exposed to 100 nM SR141716A (hatched column) or to Tween 80 (open column). Each column represents the mean value of the change in the amplitude of contractions expressed as a percentage of the amplitude measured immediately before the addition of SR141716A or Tween 80 to the organ bath ($n=32$ and 29 different bladders, respectively); vertical lines indicate s.e.mean. The asterisks indicate the significance of differences between mean amplitudes before and 30 min after addition of SR141716A or Tween 80 ($P<0.01$; Student's t test for paired data).

hydrocannabinol as a CB_1 receptor agonist, in at least one mammalian cell line (Felder *et al.*, 1995). It is noteworthy, however, that apart from WIN 55,212-2, all the cannabinoid agonists that we used in this investigation have a similar rank order of potency for inhibition of electrically-evoked bladder contractions as for the displacement of a radiolabelled probe from CB_1 binding sites (Herkenham *et al.*, 1990; Felder *et al.*, 1992; 1995; Compton *et al.*, 1993; Hillard *et al.*, 1995). Moreover, cannabinoid CB_2 receptors have so far only been found to occur naturally in cells of the immune system.

Bladder contractions evoked by electrical stimulation were almost completely abolished by tetrodotoxin, a specific Na^+ channel blocker. This suggests that the main effect of the electrical stimuli that we applied to the bladder was to provoke contractions indirectly by stimulating the release of contractile transmitters from prejunctional nerve terminals. The ability of cannabinoids to inhibit electrically-evoked bladder contractions may well depend on an ability to suppress the release of these transmitters rather than on some ability to interact directly with smooth muscle. Acetylcholine and ATP are both thought to serve as contractile transmitters in the urinary bladder of rodents (Burnstock *et al.*, 1972; Brown *et al.*, 1979; Boland *et al.*, 1993) and our experiments with mouse bladder showed that concentrations of CP 55,244 or Δ^9-tetrahydrocannabinol which had a marked depressant effect on electrically-evoked contractions did not attenuate contractions induced by acetylcholine or by the stable ATP analogue, β,γ-methylene-L-ATP.

AM 630, at a concentration of 1 μM, did not attenuate the inhibitory effects of the cannabinoid receptor agonists, Δ^9-tetrahydrocannabinol or CP 55,244, on mouse bladder. This observation contrasts with results from previous experiments with the mouse isolated vas deferens in which AM 630 was found to behave as a reasonably potent cannabinoid receptor antagonist (Pertwee *et al.*, 1995a). Nor did AM 630 affect the amplitude of electrically-evoked bladder contractions when administered by itself. This also contrasts with another previous finding, that AM 630 behaves as a cannabinoid CB_1 receptor agonist in the myenteric plexus preparation of guinea-pig small intestine (Pertwee *et al.*, 1996a). The question of why AM 630 should behave so differently in these three tissues has yet to be addressed.

In the absence of other drugs, SR141716A produced small but significant increases in the amplitude of electrically-evoked contractions. This may be because mouse bladder can itself produce a cannabinoid CB_1 receptor agonist and that this exerts an inhibitory effect on evoked contractions that can be reversed by SR141716A. Possible candidates for this endogenous cannabinoid include anandamide, although this has so far been detected only in the brain (Devane *et al.*, 1992), and 2-arachidonylglycerol which has been found peripherally, albeit so far only in canine intestine (Mechoulam *et al.*, 1995). The effect of SR141716A on twitch amplitude can also be explained in terms of a two-state receptor model (see Leff, 1995) provided it is assumed that cannabinoid receptors in mouse bladder can exist both in an activated state which can couple to the signal transduction system and in an inactivated state which cannot, that these two states are normally in equilibrium, that even when unoccupied some cannabinoid receptors in the bladder are in the activated state and that SR141716A binds preferentially to the inactivated state. If these assumptions are valid, then SR141716A would be expected to shift the equilibrium away from the activated state thereby causing a reduction in the number of precoupled receptors and hence a disinhibition of the twitch amplitude. According to this hypothesis SR141716A behaves as an inverse agonist rather than a pure antagonist in mouse bladder (see Leff, 1995). If this is true, than the K_D values of SR141716A listed in Table 2 may provide an overestimate of the real ability of this compound to bind to cannabinoid receptors in the bladder (see Kenakin, 1993).

In conclusion, the present results lend further support to the hypothesis that cannabinoid CB_1 receptors are present on peripheral nerve terminals in at least some tissues. Thus there is evidence that receptors with which cannabinoids can interact to inhibit electrically-evoked contractions are to be found not only in the mouse urinary bladder but also in the vas deferens of mouse, guinea-pig and rat and the small in-

testine of mouse and guinea-pig (Pacheco *et al.*, 1991; Paterson & Pertwee, 1993; Pertwee *et al.*, 1992; 1993; 1995b, 1996b; Rinaldi-Carmona *et al.*, 1994). For some of these tissues at least, the mouse vas deferens (Pertwee & Griffin, 1995), the guinea-pig small intestine (Pertwee, 1990; Pertwee *et al.*, 1996a) and the mouse urinary bladder (this paper), there is also evidence that this inhibitory effect is mediated by prejunctional cannabinoid CB_1 receptors that inhibit contractile transmitter release when activated. Cannabinoid receptors are also thought to be present on nerve terminals in the brain (Herkenham *et al.*, 1991). It remains to be established whether modulation of transmitter release by cannabinoid receptors is ever initiated by endogenously released cannabinoids and, therefore, of physiological significance. Also still to be established is the extent to which the ability of cannabinoids to relieve bladder spasms experienced by some patients with multiple sclerosis (see Pertwee, 1995) is mediated by cannabinoid CB_1 receptors located on the terminals of neurones innervating the bladder.

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