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Pharmacological characterization of three novel cannabinoid receptor agonists in the mouse isolated vas deferens

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Abstract

The novel compounds, 1-pentyl-2-methyl-3-(1-naphthoyl)indole, 1-pentyl-3-(1-naphthoyl)pyrrole and 1-heptyl-3-(1-naphthoyl)indole, produced a dose-related inhibition of electrically evoked contractions of the mouse vas deferens, with IC_{50} values of 2.56 nM, 3.38 nM and 639 nM respectively. K_d values of the selective CB_1 cannabinoid receptor antagonist, SR141716A [*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride], determined in the vas deferens from experiments with these compounds are 1.34 nM, 3.86 nM and 8.06 nM respectively, indicating their susceptibility to antagonism by SR141716A is similar to that of their parent compound, the CB_1 cannabinoid receptor agonist 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}. SR141716A (100 nM) had no effect on the actions of two non-cannabinoid receptor agonists, morphine and clonidine. These results provide strong support for the hypothesis that 1-pentyl-2-methyl-3-(1-naphthoyl)indole, 1-pentyl-3-(1-naphthoyl)pyrrole and 1-heptyl-3-(1-naphthoyl)indole are cannabinoid receptor agonists and confirm that the WIN 55,212-2 molecule can be modified considerably without detectable loss of cannabinoid activity.

Keywords: Cannabinoid; Cannabinoid receptor antagonist; SR141716A; Vas deferens, mouse; 1-Pentyl-2-methyl-3-(1-naphthoyl)indole; 1-Pentyl-3-(1-naphthoyl)pyrrole; 1-Heptyl-3-(1-naphthoyl)indole

1. Introduction

In the 30 years since Δ^9-tetrahydrocannabinol (3-pentyl-6*a*,7,8,10*a*-tetrahydro-6,6,9-trimethyl-6*H*-dibenzo[*b*, *d*]pyran-1-ol) was identified as the principal psychoactive component of marijuana (Gaoni and Mechoulam, 1964), a comprehensive set of structure-activity relationships has been developed (Razdan, 1986; Mechoulam et al., 1992). These relationships, which are based on the dibenzopyran structure of Δ^9-tetrahydrocannabinol (Fig. 1), require a phenolic hydroxyl group at C-1, an alkyl side chain at C-3 and appropriate substituents at C-9. Recently, several synthetic non-classical cannabinoids have been identified. Amongst these are a 3-arylcyclohexanol, CP 55,940 {(−)-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-4-(3-hydroxypropyl)cyclohexan-1-ol} (Johnson and Melvin, 1986) 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}, an aminoalkylindole (Pacheco et al., 1991). Both compounds show typical cannabinoid properties in vivo and in vitro (Pertwee, 1993). However, although it is possible to recognize a structural relationship between CP 55,940 and Δ^9-tetrahydrocannabinol that would permit them to bind to the same receptor, it is difficult to envision such a relationship between Δ^9-tetrahydrocannabinol and WIN 55,212-2 (Figs. 1 and 2a).

On the basis of molecular modelling studies, Huffman et al. (1994) developed a model in which C-7 of the naphthalene ring of WIN 55,212-2 coincided with C-9 of Δ^9-tetrahydrocannabinol and the carbonyl and morpholinoethyl groups of the aminoalkylindole were equivalent to the phenolic hydroxyl and C-3 side chain of Δ^9-tetrahydrocannabinol respectively. As a result of these studies, a series of 1-alkyl-3-(1-naphthoyl)-2-

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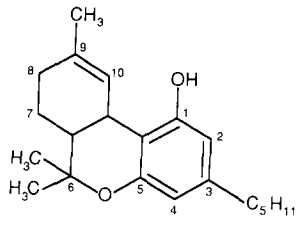

Fig. 1. Structure of Δ^9-tetrahydrocannabinol.

methylindoles were designed and synthesized and their pharmacology evaluated, both by in vitro binding and by the in vivo mouse tetrad bioassay (Huffman et al., 1994). Several of these compounds showed significant cannabinoid activity, and one of them, 1-pentyl-2-methyl-3-(1-naphthoyl)indole (Fig. 2b), was considerably more potent than Δ^9-tetrahydrocannabinol. Subsequently, it was found that indole derivatives which lack the 2-methyl substituent, for example 1-heptyl-3-(1-naphthoyl)indole (Fig. 2c), also bind to cannabinoid receptors (Huffman, Philips, Martin and Compton, unpublished). More recent modelling studies have indicated that the benzenoid portion of WIN 55,212-2 does not correspond to any portion of the Δ^9-tetrahydrocannabinol molecule and a series of 1-alkyl-3-(1-naphthoyl)pyrroles were, therefore, designed and synthesized (Lainton et al., 1995). One member of this series of compounds, 1-pentyl-3-(1-naphthoyl)pyrrole (Fig. 2d), was comparable to Δ^9-tetrahydrocannabinol both in its affinity for cannabinoid binding sites and in its potency in the mouse tetrad bioassay (Lainton et al., 1995).

The purpose of the present experiments was to investigate further the question of whether 1-pentyl-2-methyl-3-(1-naphthoyl)indole, 1-heptyl-3-(1-naphthoyl)indole and 1-pentyl-3-(1-naphthoyl)pyrrole should indeed be classified as cannabinoid receptor agonists. This objective was achieved by determining firstly whether these analogues share the ability of established cannabinoid receptor agonists to produce a dose-dependent inhibition of electrically evoked contractions of the mouse isolated vas deferens when administered at sub-micromolar concentrations (Pacheco et al., 1991; Pertwee et al., 1992, 1993) and secondly whether they can be antagonized by the selective CB_1 cannabinoid receptor 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).

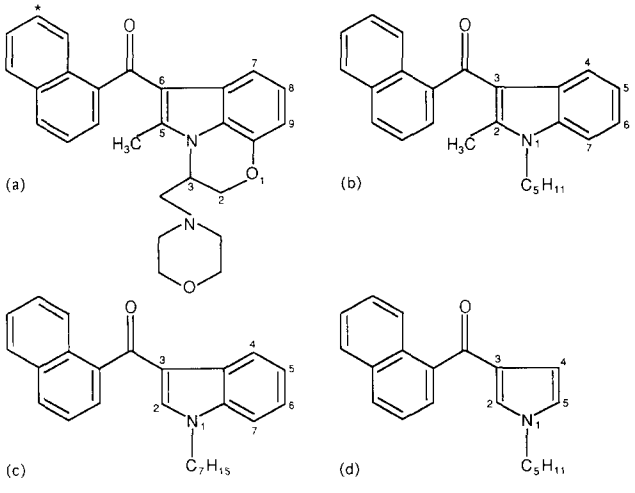

Fig. 2. Structures of (a) WIN 55,212-2, (b) 1-pentyl-2-methyl-3-(1-naphthoyl)indole, (c) 1-heptyl-3-(1-naphthoyl)indole and (d) 1-pentyl-3-(1-naphthoyl)pyrrole. C-7 of the naphthalene ring of WIN 55,212-2 has been labelled with an asterisk.

2. Materials and methods

2.1. Drugs

Δ^9-Tetrahydrocannabinol was obtained from the National Institute on Drug Abuse, CP 55,940 from Pfizer, WIN 55,212-2 from Sanofi Winthrop and SR141716A from Sanofi. The compounds 1-pentyl-2-methyl-3-(1-naphthoyl)indole, 1-heptyl-3-(1-naphthoyl)indole and 1-pentyl-3-(1-naphthoyl)pyrrole (Fig. 2) were synthesized using methods described elsewhere (Huffman et al., 1994; Lainton et al., 1995). Morphine HCl was obtained from MacFarlan Smith and clonidine HCl from Sigma. These were both dissolved in saline. 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.

2.2. Electrical stimulation

Vasa deferentia were obtained from albino MF1 mice weighing 33–62 g. Each tissue was mounted in a 4 ml organ bath at an initial tension of 0.5 g using the method described by Pertwee et al. (1993). 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. 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 electrodes attached to the upper and lower ends 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).

Each tissue 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 again stimulated. In experiments with morphine and clonidine, the second stimulation phase was continuous while in experiments with Δ^9-tetrahydrocannabinol, CP 55,940, WIN 55,212-2 and its analogues it was discontinuous, each period of stimulation lasting for 5 min. Baths were washed out by overflow at the end of each stimulation period.

2.3. Experiments without SR141716A or Tween 80 pretreatment

These experiments were performed with Δ^9-tetrahydrocannabinol, CP 55,940, WIN 55,212-2 and its analogues. 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 drug addition was 25 min for Δ^9-tetrahydrocannabinol and 10 min for the other compounds tested.

2.4. Experiments with SR141716A

SR141716A was added immediately after the 11 min stimulation period (time zero) and also after each bath wash. Δ^9-Tetrahydrocannabinol, CP 55,940, WIN 55,212-2 and its analogues were added at the end of each 5 min stimulation period, after each bath wash and subsequent addition of SR141716A. The duration of the stimulation-free period that followed each addition of agonist was 25 min for Δ^9-tetrahydrocannabinol and 10 min for the other compounds. Clonidine and morphine were first added 10–15 min after the first addition of SR141716A and then at intervals of 5 min and 8 min respectively. Baths were washed out by overflow as soon as each dose of morphine or clonidine had produced its full inhibitory effect (within 3 min).

In control experiments, Tween 80 was added instead of SR141716A. The control dose of Tween 80 was the same as the dose added in combination with the highest dose of SR141716A used (186 ng). This dose of Tween 80 (372 ng) did not significantly affect the position of the log concentration-response curve of any of the twitch inhibitors 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.

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 a twitch inhibitor with its amplitude immediately before the first addition of the inhibitor. In experiments with SR141716A, dose-response curves of CP 55,940 were constructed in the presence of more than one concentration of the antagonist and the dissociation constant (K_d) for the interaction between SR141716A and cannabinoid receptors has been 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 a twitch inhibitor that produces a particular degree of inhibition in the presence of SR141716A at a concentration, B, divided by the dose of the same twitch inhibitor that produces an identical degree of inhibition in the absence of SR141716A. Dose ratios were calculated using Allfit (National Institutes of Health, USA). The Schild slope for the interaction between CP 55,940 and SR141716A has been obtained from the best-fit straight line of a plot of $\log (x - 1)$ against $-\log B$ (Tallarida et al., 1979). The equation for this graph, $\log (x - 1) = -(-\log B) - \log K_d$, predicts a slope of -1 for all receptor-mediated interactions between agonists and antagonists that are competitive and reversible.

K_d values of SR141716A determined from experiments with twitch inhibitors other than CP 55,940 were each calculated by substituting a single dose ratio value into the above equation. Each of these dose ratio values and its 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 the slope, $1/K_d$, for the Schild slope and for agonist concentrations producing 50% inhibition of the twitch response (IC_{50} values), have been calculated by linear regression analysis using GraphPAD InPlot (GraphPAD Software, San Diego, CA, USA).

3. Results

3.1. Experiments with WIN 55,212-2 and its analogues

As shown in Fig. 3, WIN 55,212-2, 1-pentyl-2-methyl-3-(1-naphthoyl)indole, 1-pentyl-3-(1-naphthoyl)

pyrrole and 1-heptyl-3-(1-naphthoyl)indole each produced a concentration-related inhibition of electrically evoked contractions of the mouse vas deferens. The mean IC_{50} values of these drugs with their 95% confidence limits shown in brackets are respectively 1.69 nM (1.28 and 2.25 nM), 2.56 nM (1.52 and 4.32 nM), 3.38 nM (2.20 and 5.19 nM) and 639 nM (409 and 999 nM). At a concentration of 31.62 nM, SR141716A behaved as a competitive surmountable antagonist of all four of these compounds, producing parallel rightward shifts in each of their log concentration-response curves (Fig. 4). The susceptibility of the agonists, 1-pentyl-2-methyl-3-(1-naphthoyl)indole, 1-pentyl-3-(1-naphthoyl)pyrrole and 1-heptyl-3-(1-naphthoyl)indole, to antagonism by SR141716A was no different from that of their parent compound, WIN 55,212-2 (Fig. 4 and Table 1). The log concentration-response curve for

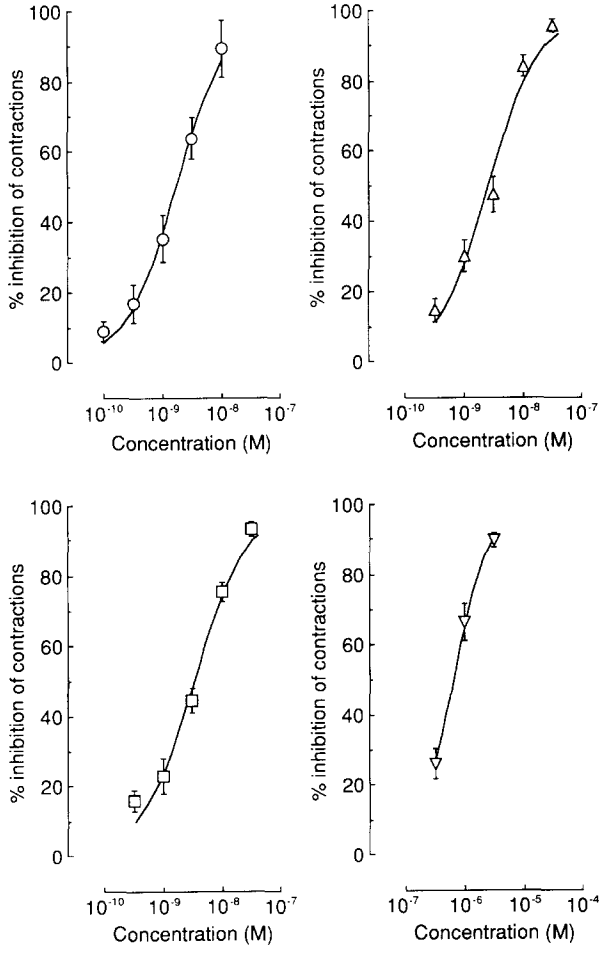

Fig. 3. Mean concentration-response curves for (a) WIN 55,212-2 (circles), (b) 1-pentyl-2-methyl-3-(1-naphthoyl)indole (triangles), (c) 1-pentyl-3-(1-naphthoyl)pyrrole (squares) and (d) 1-heptyl-3-(1-naphthoyl)indole (inverted triangles). Each symbol represents the mean value ± S.E. of inhibition of electrically evoked contractions expressed as a percentage of the amplitude of the twitch response measured immediately before the first addition of a drug to the organ bath (n = 7 or 8 different vasa deferentia).

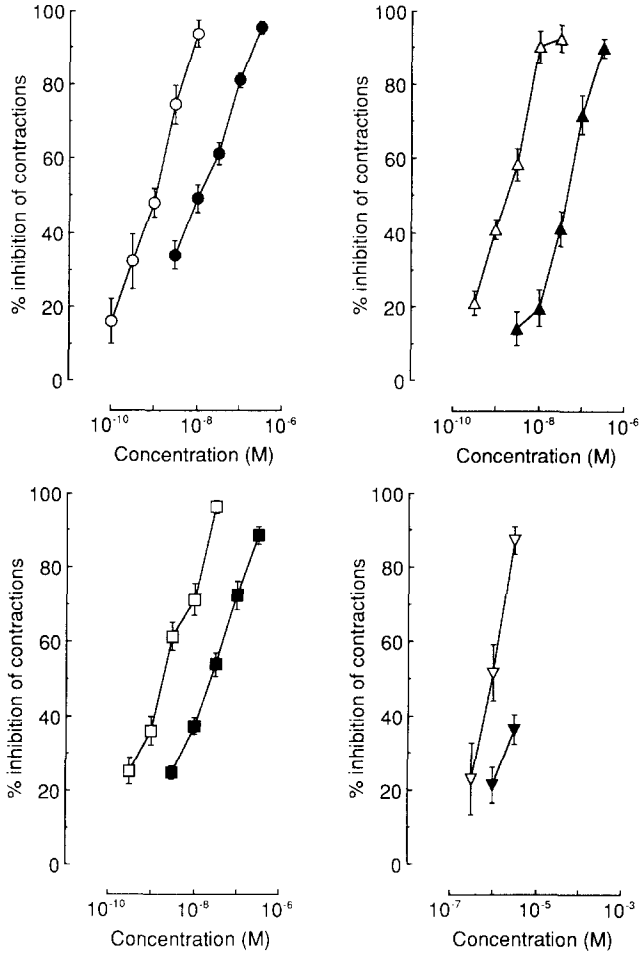

Fig. 4. Mean concentration-response curves for (a) WIN 55,212-2 (circles), (b) 1-pentyl-2-methyl-3-(1-naphthoyl)indole (triangles), (c) 1-pentyl-3-(1-naphthoyl)pyrrole (squares) and (d) 1-heptyl-3-(1-naphthoyl)indole (inverted triangles) constructed in the presence of 31.62 nM SR141716A (filled symbols) or in the presence of its vehicle, Tween 80 (open symbols). Each symbol represents the mean value ± S.E. 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 twitch inhibitor to the organ bath (n = 6–8 different vasa deferentia).

1-heptyl-3-(1-naphthoyl)indole constructed in the presence of SR141716A is incomplete as addition of concentrations above 3.16 μM would have produced Tween 80 concentrations in the assay that would themselves have inhibited the twitch response.

3.2. *Experiments with CP 55,940, Δ^9-tetrahydrocannabinol, morphine and clonidine*

K_d values of SR141716A calculated from data obtained in our experiments with WIN 55,212-2 and two of its analogues (Table 1) were markedly less than K_d values that have been obtained from previous mouse vas deferens experiments, albeit performed with other cannabinoid receptor agonists (Rinaldi-Carmona et al., 1994). We decided, therefore, to determine the K_d

Table 1
Dissociation constants (K_d) of SR141716A determined in the presence of various cannabinoid receptor agonists using the mouse vas deferens

Agonist	K_d Mean (nM)	K_d 95% confidence limits (nM)
WIN 55,212-2	2.40	1.28 and 4.53
1-Pentyl-2-methyl-3-(1-naphthoyl) indole	1.34	0.84 and 2.05
1-Pentyl-3-(1-naphthoyl)pyrrole	3.86	2.17 and 7.20
1-Heptyl-3-(1-naphthoyl)indole	8.06	3.73 and 15.32
CP 55,940	0.64 [a]	0.60 and 0.68
Δ^9-Tetrahydrocannabinol	2.66	1.43 and 4.96

[a] Determined from a Schild analysis using three concentrations of SR141716A (10, 31.62 and 100 nM). All the other K_d values were determined with a single concentration of SR141716A (31.62 nM).

value of SR141716A in the presence of two additional cannabinoid receptor agonists, Δ^9-tetrahydrocannabinol and CP 55,940. The first of these compounds was selected because it is the prototype of classical cannabinoids while CP 55,940 was included as it had been used in the earlier SR141716A experiments of Rinaldi-Carmona et al. (1994). As in these earlier experiments, we measured the ability of several concentrations of SR141716A to antagonize CP 55,940. This allowed use of Schild analysis to test whether SR141716A does indeed behave as a competitive antagonist (Rinaldi-Carmona et al., 1994). To explore its selectivity as a cannabinoid receptor antagonist in the vas deferens, we also determined whether SR141716A would block the inhibitory effects on this tissue of two

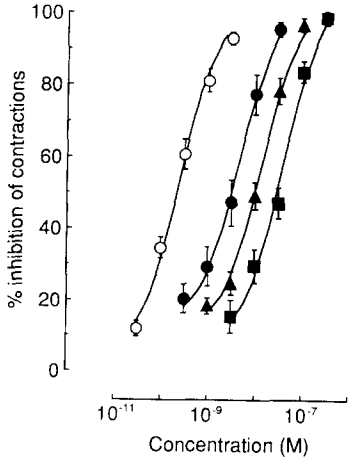

Fig. 5. Mean concentration-response curves for CP 55,940 constructed in the presence of 10 nM (filled circles), 31.62 nM (filled triangles) or 100 nM SR141716A (filled squares) or in the presence of Tween 80 (open circles). Each symbol represents the mean value ± S.E. of inhibition of electrically evoked contractions 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 ($n = 7$ or 8 different vasa deferentia).

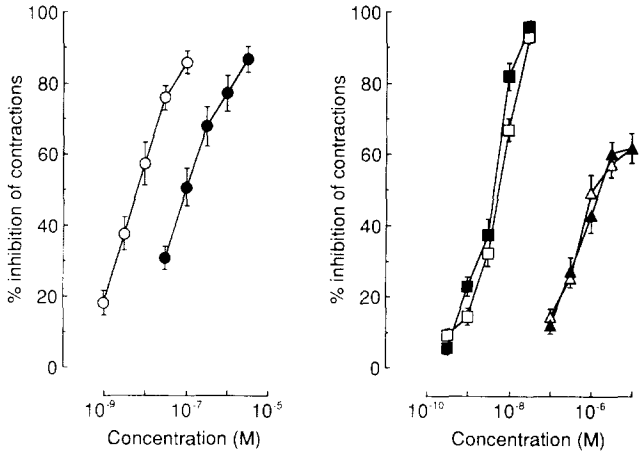

Fig. 6. Mean concentration-response curves for Δ^9-tetrahydrocannabinol (circles), clonidine (squares) and morphine (triangles) constructed in the presence of SR141716A (filled symbols) or Tween 80 (open symbols). The concentration of SR141716A was either 31.62 nM (Δ^9-tetrahydrocannabinol experiment) or 100 nM (morphine and clonidine experiments). Each symbol represents the mean value ± S.E. 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 twitch inhibitor to the organ bath ($n = 6$–8 different vasa deferentia).

non-cannabinoid twitch inhibitors, morphine and clonidine.

SR141716A produced parallel rightward shifts in the log concentration-response curves of CP 55,940 and Δ^9-tetrahydrocannabinol, the degree of shift to the right depending on the dose of SR141716A used (Figs. 5 and 6). As shown in Table 1, the K_d value of SR141716A calculated from data obtained in our experiments with Δ^9-tetrahydrocannabinol was the same as that determined in our earlier experiments with WIN 55,212-2. The K_d value calculated from the CP 55,940 data was even less than this. The Schild slope for the interaction between CP 55,940 and SR141716A was estimated to be −0.93 with 95% confidence limits of −0.75 and −1.12.

The highest concentration of SR141716A used in the present experiments (100 nM) antagonized neither morphine nor clonidine (Fig. 6). None of the concentrations of SR141716A or Tween 80 used in this investigation had any detectable effect on the amplitude of the twitch response (data not shown).

4. Discussion

The present experiments showed that each of the three analogues of WIN 55,212-2 that we investigated shares the ability of sub-micromolar concentrations of known CB_1 cannabinoid receptor agonists (Pertwee, 1993) to produce a dose-related inhibition of electrically evoked contractions of the mouse vas deferens.

Our experiments also demonstrated that the inhibitory effects of these analogues could be significantly attenuated by the CB_1 cannabinoid receptor antagonist, SR141716A. As in previous experiments with the mouse vas deferens (Rinaldi-Carmona et al., 1994), SR 141716A behaved as a competitive antagonist, Thus it produced parallel rightward shifts in the log concentration-response curves of Δ^9-tetrahydrocannabinol, CP 55,940, WIN 55,212-2 and its analogues and the Schild slope for the interaction between CP 55,940 and SR141716A was found not to differ significantly from −1. SR141716A showed essentially the same potency in antagonizing 1-pentyl-2-methyl-3-(1-naphthoyl)indole, 1-heptyl-3-(1-naphthoyl)indole and 1-pentyl-3-(1-naphthoyl)pyrrole as in antagonizing their parent compound, WIN 55,212-2, supporting the hypothesis that these compounds are interacting with the same population of receptors. Presumably these are CB_1 cannabinoid receptors as there is evidence from its binding properties that SR141716A is highly selective as a CB_1 receptor ligand (Rinaldi-Carmona et al., 1994) and as a concentration of SR141716A capable of markedly antagonizing cannabinoid-induced inhibition of the twitch response of the vas deferens (100 nM) was found in the present investigation to have no effect on the ability of two non-cannabinoid receptor agonists, morphine and clonidine, to inhibit this response.

The degree of antagonism produced by SR141716A has been expressed in terms of its dissociation constant, K_d. When the value of this constant was calculated from results obtained in our experiments with CP 55,940, it was found to be 0.64 nM. This is in good agreement with the K_i value of SR141716A (1.98 nM) determined from its ability to displace CP 55,940 from cannabinoid binding sites (Rinaldi-Carmona et al., 1994). However, it is markedly less than the K_d value of SR141716A obtained by Rinaldi-Carmona et al. (1994) in their vas deferens experiments with CP 55,940 (10.47 nM), indicating that SR141716A may be even more potent as a cannabinoid receptor antagonist than was originally thought. We have also found the IC_{50} of CP 55,940 for inhibition of electrically evoked contractions of the mouse vas deferens (0.2 nM) (Fig. 5; see also Pertwee, 1993) to be much less than the IC_{50} value of CP 55,940 (3.9 nM) obtained by Rinaldi-Carmona et al. (1994). It is worth noting, therefore, that there are a number of differences between the ways in which our experiments and those of Rinaldi-Carmona et al. (1994) were carried out. In particular, the delay between the addition of SR141716A and CP 55,940 was far less in the present experiments (15 min) than in the previous study (60 min). In addition, we used a different strain of mice and also a different kind of drug vehicle for SR141716A and CP 55,940: a non-ionic surfactant (Tween 80) rather than an organic solvent (dimethyl sulfoxide). Further experiments would be required to determine whether any of these differences are responsible for the discrepancies between our data and those of Rinaldi-Carmona et al. (1994).

The results obtained show that 1-pentyl-2-methyl-3-(1-naphthoyl)indole is significantly more potent than 1-heptyl-3-(1-naphthoyl)indole as an inhibitor of the twitch response. This is in accord with the relative affinities of these two compounds for cannabinoid binding sites (Huffman, Philips, Martin and Compton, unpublished). However, 1-pentyl-3-(1-naphthoyl)pyrrole has been shown to bind less avidly to cannabinoid binding sites than 1-pentyl-2-methyl-3-(1-naphthoyl)indole (Huffman et al., 1994; Lainton et al., 1995) and yet these compounds are equipotent as inhibitors of the twitch response. The reason for this lack of correlation between binding affinity and pharmacological activity remains to be determined.

In conclusion, our results provide strong support for the hypothesis that 1-pentyl-2-methyl-3-(1-naphthoyl) indole, 1-heptyl-3-(1-naphthoyl)indole and 1-pentyl-3-(1-naphthoyl)pyrrole are cannabinoid receptor agonists. The present findings also confirm that the WIN 55,212-2 molecule can be modified considerably without detectable loss of cannabinoid activity. Thus, as predicted from the molecular modelling studies of Huffman et al. (1994), the ability to inhibit electrically evoked contractions of the vas deferens was found to be unaffected both by replacement of the two morpholine rings of WIN 55,212-2 with a pentyl group to yield 1-pentyl-2-methyl-3-(1-naphthoyl)indole and by additional modification of the indole nucleus through removal of the aromatic ring and of the methyl group at the 2 position to yield 1-pentyl-3-(1-naphthoyl)pyrrole.

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References

Colquhoun, D., 1971, Lectures on Biostatistics (Oxford University Press, Oxford).

Gaoni, Y. and R. Mechoulam, 1964, Isolation, structure and partial synthesis of an active constituent of hashish, J. Am. Chem. Soc. 86, 1646.

Huffman, J.W., D. Dai, B.R. Martin and D.R. Compton, 1994, Design, synthesis and pharmacology of cannabimimetic indoles, BioMed. Chem. Lett. 4, 563.

Johnson, M.R. and L.S. Melvin, 1986, The discovery of nonclassical cannabinoid analgetics, in: Cannabinoids as Therapeutic Agents, ed. R. Mechoulam (CRC Press, Boca Raton) p. 121.

Lainton, J.A.H., J.W. Huffman, B.R. Martin and D.R. Compton, 1995, 1-Alkyl-3-(1-naphthoyl)pyrroles: a new class of cannabinoid, Tetrahedron Lett. 36, 1401.

Mechoulam, R., W.A. Devane and R. Glaser, 1992, Cannabinoid geometry and biological activity, in: Marijuana/Cannabinoids. Neurobiology and Neurophysiology, eds. L. Murphy and A. Bartke (CRC Press, Boca Raton) p. 1.

Pacheco, M., S.R. Childers, R. Arnold, F. Casiano and S.J. Ward, 1991, Aminoalkylindoles: actions on specific G-protein-linked receptors, J. Pharmacol. Exp. Ther. 257, 170.

Pertwee, R., 1993, The evidence for the existence of cannabinoid receptors, Gen. Pharmacol. 24, 811.

Pertwee, R.G., L.A. Stevenson, D.B. Elrick, R. Mechoulam and A.D. Corbett, 1992, Inhibitory effects of certain enantiomeric cannabinoids in the mouse vas deferens and the myenteric plexus preparation of guinea-pig small intestine, Br. J. Pharmacol. 105, 980.

Pertwee, R.G., L.A. Stevenson and G. Griffin, 1993, Cross-tolerance between delta-9-tetrahydrocannabinol and the cannabimimetic agents, CP 55,940, WIN 55,212-2 and anandamide, Br. J. Pharmacol. 110, 1483.

Razdan, R.K., 1986, Structure-activity relationships in cannabinoids, Pharmacol. Rev. 38, 75.

Rinaldi-Carmona, M., F. Barth, M. Héaulme, D. Shire, B. Calandra, C. Congy, S. Martinez, J. Maruani, G. Néliat, D. Caput, P. Ferrara, P. Soubrié, J.C. Brelière and G. Le Fur, 1994, SR141716A, a potent and selective antagonist of the brain cannabinoid receptor, FEBS Lett. 350, 240.

Tallarida, R.J., A. Cowan and A.W. Adler, 1979, pA_2 and receptor differentiation: a statistical analysis of competitive antagonism, Life Sci. 25, 637.