*Br. J. Pharmacol.* (1993), **110**, 1483–1490 

# Cross-tolerance between delta-9-tetrahydrocannabinol and the cannabimimetic agents, CP 55,940, WIN 55,212-2 and anandamide

[1]R.G. Pertwee, L.A. Stevenson & G. Griffin

Department of Biomedical Sciences, Marischal College, University of Aberdeen, Aberdeen AB9 1AS

**1** Mice pretreated intraperitoneally for 2 days with delta-9-tetrahydrocannabinol (delta-9-THC) at a dose of 20 mg $kg^{-1}$ $day^{-1}$ and then challenged intravenously with this drug, 24 h after the second pretreatment, showed a 6 fold tolerance to the hypothermic effect of delta-9-THC. This pretreatment also induced tolerance to the hypothermic effects of the cannabimimetic agents, CP 55,940 (4.6 fold) and WIN 55,212-2 (4.9 fold), but not to the hypothermic effect of the putative endogenous cannabinoid, anandamide.

**2** Vasa deferentia removed from mice pretreated intraperitoneally with delta-9-THC twice at a dose of 20 mg $kg^{-1}$ $day^{-1}$ were less sensitive to its inhibitory effect on electrically-evoked contractions than vasa deferentia obtained from control animals. The cannabinoid pretreatment induced a 30 fold parallel rightward shift in the lower part of the concentration-response curve of delta-9-THC and a marked reduction in the maximal inhibitory effect of the drug. It also induced tolerance to the inhibitory effects on the twitch response of CP 55,940 (8.7 fold), WIN 55,212-2 (9.6 fold) and anandamide (12.3 fold).

**3** The results confirm that cannabinoid tolerance can be rapid in onset and support the hypothesis that it is mainly pharmacodynamic in nature. The finding that *in vivo* pretreatment with delta-9-THC can produce tolerance not only to its own inhibitory effect on the vas deferens but also to that of three other cannabimimetic agents, suggests that this tissue would be suitable as an experimental model for investigating the mechanisms responsible for cannabinoid tolerance.

**4** Further experiments are required to establish why tolerance to anandamide-induced hypothermia was not produced by a pretreatment with delta-9-THC that did induce tolerance to the hypothermic effects of delta-9-THC, CP 55,940 and WIN 55,212-2 and to the inhibitory effects of delta-9-THC, CP 55,940, WIN 55,212-2 and anandamide on the twitch response of the vas deferens.

**Keywords:** Delta-9-tetrahydrocannabinol; CP 55,940; WIN 55,212-2; anandamide; cannabinoids; cannabinoid tolerance; mouse vas deferens; hypothermia

## Introduction

Little is known about the mechanisms underlying the development of tolerance to psychotropic cannabinoids other than that they are largely pharmacodynamic in nature (Pertwee, 1991). As a first step aimed at identifying these mechanisms, the present investigation set out to establish whether *in vivo* pretreatment with delta-9-tetrahydrocannabinol (delta-9-THC) can induce tolerance to the inhibitory effect of this drug on electrically-evoked contractions of the mouse isolated vas deferens, our overall purpose being to explore the suitability of this preparation as a model with which to determine the basis of cannabinoid tolerance. The mouse vas deferens was used as there is already evidence that it is an appropriate tissue for investigating the mechanisms responsible for the acute effects of psychotropic cannabinoids. In particular, cannabimimetic agents are known to be highly potent and to show remarkable stereoselectivity as inhibitors of the electrically-evoked twitch response of this tissue (Pertwee *et al.*, 1992a; 1993). It is also known that the potency of cannabinoids as inhibitors of the twitch response correlates well with their potency as psychotropic agents (Pertwee, 1993).

The ability of an *in vivo* pretreatment with delta-9-THC to induce tolerance to the effects of other cannabimimetic agents on the twitch response of the mouse vas deferens was also investigated. These experiments were carried out with CP 55,940, WIN 55,212-2 and the putative endogenous cannabinoid, anandamide (Martin *et al.*, 1991; Devane *et al.*, 1992). The structures of these cannabimimetic agents are shown in Figure 1. It was decided that in the first experiments at least, the delta-9-THC pretreatment used should be one that produces tolerance to delta-9-THC-induced hypothermia, previous experiments in this laboratory having shown that such tolerance can develop quite rapidly (Fitton & Pertwee, 1982). Accordingly, our initial experiments were directed at establishing a reliable procedure for producing tolerance to the hypothermic effect of delta-9-THC in mice.

Some of the results described in this paper have been presented to the British Pharmacological Society (Griffin & Pertwee, 1993).

## Methods

### *Animals*

Experiments were performed with male albino MF1 mice weighing 23 to 30 g (body temperature experiments) or 36 to 59 g (vas deferens experiments). The animals received food and water *ad libitum*. Except when stated otherwise, *in vivo* experiments were carried out at ambient temperatures of 20 to 22°C.

### *Production of tolerance*

Tolerance was induced by injecting delta-9-THC intraperitoneally at doses of up to 20 mg $kg^{-1}$ either once or twice at an interval of 24 h. Control animals received intraperitoneal injections of Tween 80. The volume for intraperitoneal injection was 0.25 ml 25 $g^{-1}$. The degree of tolerance produced by delta-9-THC was determined 24 h after the final injection either by measuring the hypothermic effects of delta-9-THC, CP 55,940, WIN 55,212-2 or anandamide or by measuring

[1] Author for correspondence.

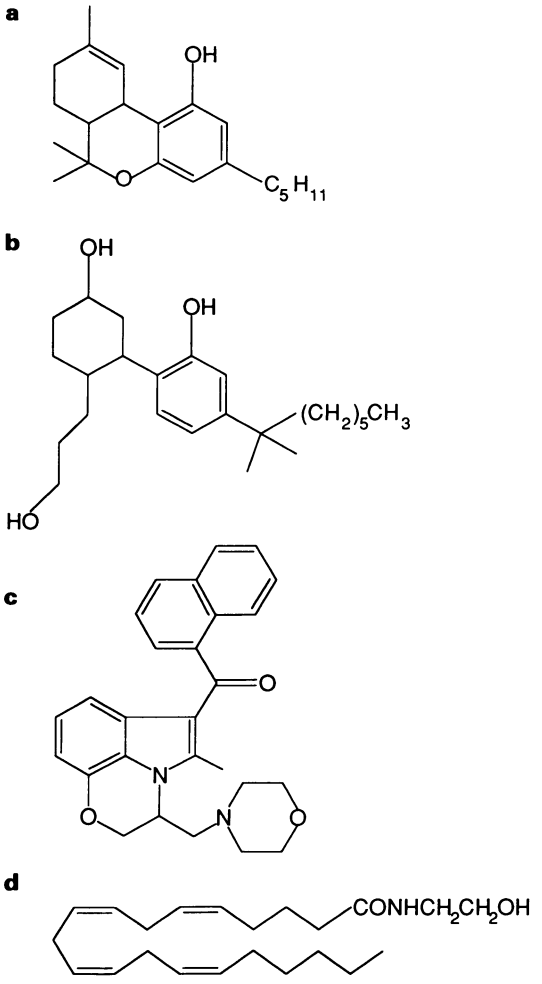


**Figure 1** The chemical structures of (a) delta-9-tetrahydrocannabinol (delta-9-THC), (b) CP 55,940, (c) WIN 55,212-2 and (d) anandamide.

the inhibitory effects of these compounds on electrically-evoked contractions of the mouse vas deferens.

*Body temperature experiments*

Body temperature was measured with a thermistor probe (YSI 402) inserted 3 cm into the rectum. Except when they were being injected intravenously or being held in the hand for the measurement of body temperature, mice were kept unrestrained, each in a separate cage. Temperature readings were first taken 30 min and 5 min before drug administration which was at time zero. Readings were next taken 5 min after time zero, then at 5 min intervals until + 30 min and finally at + 45 and + 60 min. To produce hypothermia, drugs were injected into a lateral tail vein. The injection volume was 0.2 ml 25 $g^{-1}$. The injection procedure was facilitated by subjecting the mice to an ambient temperature of 34°C for the 5 min period immediately before injection so as to cause dilatation of the tail veins. Exposure of a group of six mice to this ambient temperature for 5 min did not produce any significant change in body temperature ($P > 0.05$; Student's $t$ test for paired data), the group's mean rectal temperature being 37.05 ± 0.12°C just before the start of the exposure and 37.12 ± 0.17°C at the end.

*Vas deferens experiments*

Vasa deferentia were mounted in 4 ml organ baths, at an initial tension of 0.5 g. The baths contained $Mg^{2+}$-free Krebs solution kept at 37°C and bubbled with 95% $O_2$ and 5% $CO_2$. Isometric contractions were evoked by electrical field stimulation through a platinum electrode attached to the upper end of each bath and a stainless steel electrode attached to the lower end. The stimuli were generated by a Grass S48 stimulator, then amplified (Med-Lab channel attenuator) and finally divided to yield separate outputs to four organ baths (Med-Lab Stimusplitter). Contractions were registered on a computer (Apple Macintosh LC) using a data recording and analysis system (MacLab) that was linked via preamplifiers (Macbridge) to Pye Ether UF1 transducers. Tissues were stimulated with 0.5 s trains of three pulses of 110% maximal voltage (train frequency 0.1 Hz; pulse duration 0.5 ms). In most experiments each tissue was used to construct a single cumulative concentration-response curve and was subjected to five or six periods of stimulation. The first of these began after the tissue had equilibrated but before drug administration and continued for 11 min. Drug addition (10 µl) was made immediately after this first stimulation period. Subsequent stimulation periods lasted 5 min at the end of which the bath contents were washed out by overflow and another dose of drug added. Each 5 min stimulation period was started only after sufficient time had elapsed for the drug under investigation to achieve its full inhibitory effect. Results from pilot experiments indicated this to be 15 min for anandamide and CP 55,940, 20 min for WIN 55,212-2 and 25 min for delta-9-THC. In our first experiments (with delta-9-THC), tissues were exposed to just one concentration of drug (10 nM) and results from these experiments are also described below.

*Drugs*

Delta-9-THC was obtained from the National Institute on Drug Abuse, U.S.A., CP 55,940 from Dr L.S. Melvin (Pfizer) and WIN 55,212-2 from Dr S.J. Ward (Sterling Winthrop). Anandamide was synthesized and supplied by Professor R. Mechoulam (University of Jerusalem). Each compound was mixed with 2 parts of Tween 80 by weight and dispersed in 0.9% w/v NaCl solution (saline) as described previously for delta-9-THC (Pertwee *et al.*, 1992a).

*Analysis of data*

Values are expressed as means and limits of error as standard errors. The effect of delta-9-THC on body temperature was calculated by subtracting rectal temperature, measured 5 min before drug administration, from rectal temperature measured at the time of peak hypothermia. Inhibition of the twitch response is expressed in percentage terms and has been calculated by comparing the amplitude of the twitch response immediately before injection with its amplitude during each of the postinjection periods of stimulation. Degrees of tolerance to delta-9-THC, CP 55,940, WIN 55,212-2 and anandamide were determined by symmetrical (2 + 2) dose parallel line assays (Colquhoun, 1971). The significance of differences between means was evaluated by Dunnett's test (Dunnett, 1964) or by Student's $t$ test (two-tail) for paired or unpaired data ($P >$ or $< 0.05$).

## Results

*Tolerance to the hypothermic effect of delta-9-THC*

As shown in Figure 2, delta-9-THC induced dose-related decreases in rectal temperature. This ability of delta-9-THC to induce hypothermia was significantly attenuated by pretreating animals with the drug twice at a dose of 20 mg $kg^{-1}$ i.p., this pretreatment producing a 6 fold parallel rightward shift in the log dose-hypothermic response curve of the drug (Figure 3 and Table 1).

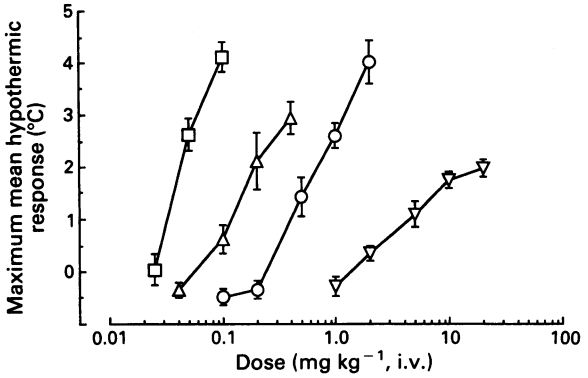


**Figure 2** Mean dose-hypothermic response curves for CP 55,940 (□), WIN 55,212-2 (△), delta-9-tetrahydrocannabinol (○), and anandamide (▽) in mice. Each symbol represents the mean value ± s.e.mean of the maximum hypothermic response produced ($n = 6$).

The degree of tolerance that was observed following intraperitoneal pretreatment with delta-9-THC was influenced both by the size of the pretreatment dose used and by the number of pretreatments given. Whereas the hypothermic response to a submaximal dose of delta-9-THC (1.0 mg kg$^{-1}$, i.v.) was abolished in mice that had been subjected to two intraperitoneal injections of this drug at a dose of 20 mg kg$^{-1}$ (Figure 4) it was only attenuated, albeit significantly, in mice that had been pretreated either with a single injection of the 20 mg kg$^{-1}$ dose or with two injections of either 5 or 10 mg kg$^{-1}$ i.p. (Table 2). Delta-9-THC pretreatment with a single injection of 10 mg kg$^{-1}$ i.p. or with two injections of 1 or 2 mg kg$^{-1}$ i.p. did not induce significant tolerance (Table 2). Maximum hypothermia usually occurred sooner after intravenous injection of delta-9-THC in drug pretreated mice than in animals that had been pretreated with Tween 80.

Some of the intraperitoneal doses of delta-9-THC used in the present experiments (10 and 20 mg kg$^{-1}$) are known to produce marked hypothermia in mice (Pertwee & Tavendale, 1977; Gray *et al.*, 1987). It was of interest, therefore, to establish whether tolerance to the hypothermic effect of

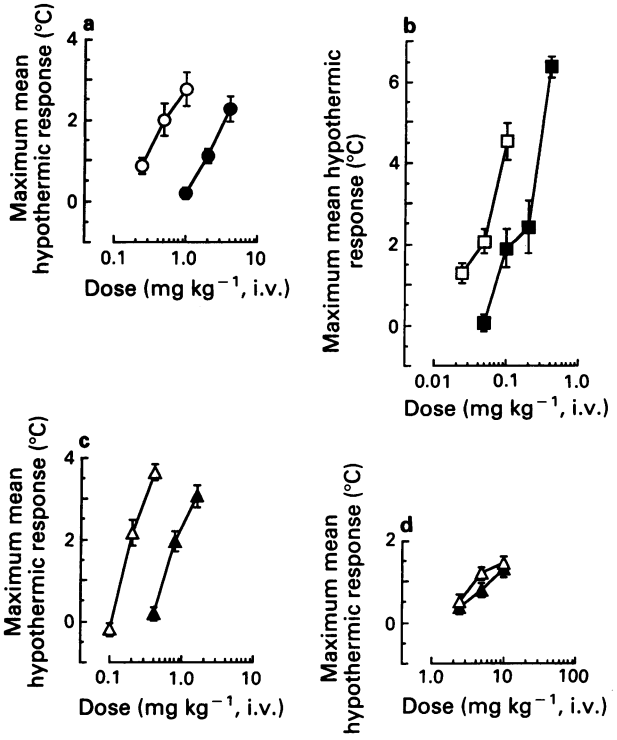


**Figure 3** Mean dose-hypothermic response curves for (a) delta-9-tetrahydrocannabinol, (b) CP 55,940, (c) WIN 55,212-2 and (d) anandamide in mice pretreated once daily for 2 days with delta-9-THC at a dose of 20 mg kg$^{-1}$, i.p. (closed symbols) or with Tween 80 at a dose of 40 mg kg$^{-1}$, i.p. (open symbols). Each symbol represents the mean value ± s.e.mean of the maximum hypothermic response produced ($n = 6$).

delta-9-THC would still develop in animals that were prevented from experiencing any significant degree of delta-9-THC-induced hypothermia during the pretreatment period. Accordingly, one series of experiments was conducted in which mice were transferred to a chamber maintained at an

**Table 1** Effect of *in vivo* pretreatment with delta-9-tetrahydrocannabinol (delta-9-THC) on the potencies of certain cannabimimetic drugs as hypothermic agents

| *Pretreatment drug* | *Pretreatment dose* (mg kg$^{-1}$, i.p.) | *Treatment drug* | *Treatment doses* (mg kg$^{-1}$, i.v.) | *Potency ratio* | *95% Confidence limits* | n |
|---|---|---|---|---|---|---|
| Delta-9-THC | 20 | Delta-9-THC | 1.0, 4.0 | 6.03 | 3.84 and 10.41 | 6 |
| Tween | 40 | Delta-9-THC | 0.25, 1.0 | | | |
| Delta-9-THC | 20 | CP 55,940 | 0.05, 0.2 | 4.57 | 2.83 and 9.09 | 6 |
| Tween | 40 | CP 55,940 | 0.025, 0.1 | | | |
| Delta-9-THC | 20 | WIN 55,212-2 | 0.8, 1.6 | 4.94 | 3.75 and 7.04 | 6 |
| Tween | 40 | WIN 55,212-2 | 0.2, 0.4 | | | |
| Delta-9-THC | 20 | Anandamide | 2.5, 10 | 1.24 | 0.77 and 2.14 | 6 |
| Tween | 40 | Anandamide | 2.5, 10 | | | |
| None | – | Delta-9-THC | 0.5, 2.0 | 1.14 | 0.59 and 1.89 | 6 |
| Tween | 40 | Delta-9-THC | 0.25, 1.0 | | | |

Mice were either unpretreated or pretreated intraperitoneally, once daily for 2 days, with delta-9-THC or with Tween 80. In the experiments in which mice were pretreated, the effects of treatment drugs, delta-9-THC, CP 55,940, WIN 55,212-2 and anandamide, on body temperature were measured 24 h after the second intraperitoneal injection. The final potency ratio listed in the Table indicates the extent by which the potency of delta-9-THC as a hypothermic agent was greater or less in unpretreated animals than in animals that had been pretreated with Tween 80. Pretreatment with Tween 80 did not lead to any significant change in the slope of the dose-response curve of delta-9-THC. Each of the other potency ratios listed above indicates the extent by which pretreatment with delta-9-THC reduced the potency of a treatment drug as a hypothermic agent.None of the delta-9-THC-induced rightward shifts in the dose-response curves of the treatment drugs deviated significantly from parallelism. Potency ratios, confidence limits and deviations of pairs of log dose-response curves from parallelism have been determined using symmetrical (2 + 2) dose parallel line assays and were calculated from data obtained with the treatment doses shown (Colquhoun, 1971).

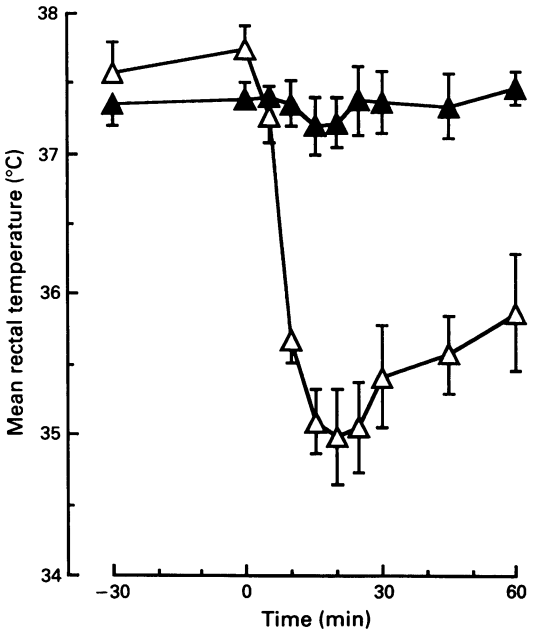


**Figure 4** Effect of pretreatment with delta-9-tetrahydrocannabinol (delta-9-THC, ▲) or Tween 80 (△) on the ability of delta-9-THC to produce hypothermia. Mice received delta-9-THC (20 mg kg$^{-1}$, i.p.) or Tween 80 (40 mg kg$^{-1}$, i.p.) once daily for 2 days. All animals were challenged with an intravenous injection of delta-9-THC (1.0 mg kg$^{-1}$) at time zero, 24 h after the second pretreatment. Each symbol represents a mean value ± s.e.mean of rectal temperature ($n = 6$). It was assumed that rectal temperature at time zero was the same as rectal temperature at − 5 min (see Methods). In the Tween-pretreated group of animals, mean rectal temperatures were significantly less than the corresponding time zero value from + 10 min onwards ($P < 0.01$; Dunnett's test). In the delta-9-THC-pretreated group, no significant changes in mean rectal temperature occurred between time zero and + 60 min ($P > 0.05$; Dunnett's test).

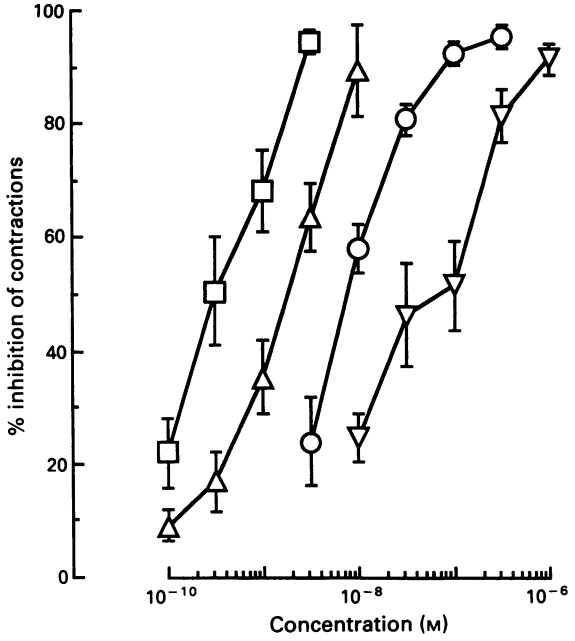


**Figure 5** Mean cumulative concentration-response curves for CP 55,940 (□), WIN 55,212-2 (△), delta-9-tetrahydrocannabinol (delta-9-THC) (○), and anandamide (▽) in mouse isolated vasa deferentia. Each symbol represents the mean value ± s.e.mean of inhibition of electrically-evoked contractions expressed as a percentage of the amplitude of the twitch response measured immediately before the first addition of drug to the organ bath ($n = 7$ to 8 different vasa deferentia).

ambient temperature of 34°C immediately after each of two pretreatment injections with delta-9-THC (20 mg kg$^{-1}$, i.p.) or Tween 80. This ambient temperature is a 'thermoneutral' temperature for mice and was used as it can prevent delta-9-THC from causing mouse rectal temperature to fall below 37°C (Pertwee & Tavendale, 1977). The mice were kept at 34°C for 3 h. This was because, in mice kept at normal room temperature, the hypothermic effect of the pretreatment dose of delta-9-THC used (20 mg kg$^{-1}$, i.p.) is known to last for this length of time (Gray *et al.*, 1987). As shown in Table 2, the degree of tolerance to delta-9-THC-induced hypothermia was little different in mice that had been kept at 34°C during part of the pretreatment period than in animals kept at normal room temperature throughout this period.

### *Tolerance to the inhibitory effect of delta-9-THC on the electrically-evoked twitch response of the mouse vas deferens*

Delta-9-THC induced a concentration-dependent inhibition of the twitch response (Figure 5). Vasa deferentia removed from mice that had been pretreated twice with delta-9-THC at doses of 10 or 20 mg kg$^{-1}$ day$^{-1}$ i.p. were significantly less sensitive to the inhibitory effects of a submaximal concentration of this drug on electrically-evoked contractions (10 nM) than tissues obtained from control animals that had been pretreated with Tween 80 (20 or 40 mg kg$^{-1}$ day$^{-1}$, i.p.). The inhibitory effect of delta-9-THC was 5.36 ± 5.1% in tissue taken from mice pre-treated with the higher dose of drug and 42.44 ± 5.49% in tissue removed from animals pretreated with the higher dose of vehicle ($n = 8$). In tissues obtained from mice pretreated with the lower dose of drug or vehicle, the inhibitory effects of delta-9-THC were respectively 27.6 ± 6.53% ($n = 7$) and 53.87 ± 7.06% ($n = 8$).

Tolerance to the inhibitory effect of delta-9-THC on the twitch response could also be produced by single drug pretreatment. Thus 10 nM delta-9-THC produced significantly less inhibition of the twitch response in vasa deferentia obtained from mice pretreated once with this drug at a dose of 20 mg kg$^{-1}$, i.p. (26.77 ± 6.46%; $n = 7$) than in tissues obtained from animals pretreated once with Tween 80 at a dose of 40 mg kg$^{-1}$, i.p. (47.09 ± 6.07%; $n = 6$). The same concentration of delta-9-THC was also significantly less inhibitory in vasa deferentia obtained from mice pretreated once with the drug at a dose of 10 mg kg$^{-1}$, i.p. (32.75 ± 4.91%; $n = 8$) than in tissues obtained from animals that had received a single injection of Tween 80 at a dose of 20 mg kg$^{-1}$, i.p. (53.01 ± 5.22%; $n = 8$).

Experiments directed at establishing the degree of tolerance induced in the vas deferens by intraperitoneal pretreatment with delta-9-THC over 2 days, showed that pretreatment with a dose of 20 mg kg$^{-1}$ day$^{-1}$ produced a marked decrease in the maximum inhibitory effect of delta-9-THC (Figure 6). There was no significant change in the slope of the lower part of the log concentration-response curve of the drug which underwent a 30 fold shift to the right (Table 3). In tissue obtained from mice pretreated twice with delta-9-THC at doses of 5 or 10 mg kg$^{-1}$, i.p., the log concentration-response curve of the drug was similarly affected, albeit to a smaller extent (Figure 6 and Table 3). Pretreatment with delta-9-THC at a dose of 2 mg kg$^{-1}$ day$^{-1}$ also produced significant tolerance. The degree of the tolerance produced by this pretreatment was less than that produced by doses of 5 or 10 mg kg$^{-1}$ day$^{-1}$ and the size of the maximum response remained unaffected (Figure 6 and Table 3).

No significant difference was observed between the amplitudes of contractions produced by electrical stimulation in tissue obtained from mice pretreated with delta-9-THC and those of contractions evoked in tissues obtained from vehicle-

**Table 3** Effect of *in vivo* pretreatment with delta-9-tetrahydrocannabinol (delta-9-THC) on the potencies of certain cannabimimetic drugs as inhibitors of the electrically-evoked twitch response of the mouse isolated vas deferens

| *Pretreatment drug* | *Pretreatment dose* (mg kg$^{-1}$, i.p.) | *Treatment drug* | *Treatment concentrations* (nM) | *Potency ratio* | *95% Confidence limits* | n |
|---|---|---|---|---|---|---|
| Delta-9-THC | 2 | Delta-9-THC | 10, 100 | 2.62 | 1.11 and 5.86 | 8 |
| Tween | 40 | Delta-9-THC | 3.16, 31.6 | | | |
| Delta-9-THC | 5 | Delta-9-THC | 31.6, 316 | 7.42 | 2.17 and 21.88 | 8 |
| Tween | 40 | Delta-9-THC | 3.16, 31.6 | | | |
| Delta-9-THC | 10 | Delta-9-THC | 31.6, 100 | 13.35 | 5.32 and 55.78 | 8 |
| Tween | 40 | Delta-9-THC | 3.16, 10 | | | |
| Delta-9-THC | 20 | Delta-9-THC | 31.6, 100 | 30.01 | 17.46 and 83.30 | 12 |
| Tween | 40 | Delta-9-THC | 3.16, 10 | | | |
| Delta-9-THC | 20 | CP 55,940 | 3.16, 31.6 | 8.74 | 4.30 and 17.33 | 6 |
| Tween | 40 | CP 55,940 | 0.316, 3.16 | | | |
| Delta-9-THC | 20 | WIN 55,212-2 | 3.16, 31.6 | 9.62 | 5.78 and 15.95 | 10 |
| Tween | 40 | WIN 55,212-2 | 0.316, 3.16 | | | |
| Delta-9-THC | 20 | Anandamide | 100, 1000 | 12.3 | 6.38 and 24.59 | 6 |
| Tween | 40 | Anandamide | 10, 100 | | | |
| None | - | Delta-9-THC | 3.16, 31.6 | 0.94 | 0.41 and 2.15 | 8 |
| Tween | 40 | Delta-9-THC | 3.16, 31.6 | | | |

Mice were either unpretreated or pretreated intraperitoneally, once daily for 2 days, with delta-9-THC or with Tween 80. In the experiments in which mice were pretreated, the effects of treatment drugs, delta-9-THC, CP 55,940, WIN 55,212-2 and anandamide, on the twitch response of the vas deferens were measured measured 24 h after the second intraperitoneal injection. The final potency ratio listed in the Table indicates the extent by which the potency of delta-9-THC as an inhibitor of the twitch response was greater or less in tissues from unpretreated animals than in tissues from animals that had been pretreated with Tween 80. Pretreatment with Tween 80 did not lead to any significant change in the slope of the concentration-response curve of delta-9-THC. Each of the other potency ratios listed above indicates the extent by which pretreatment with delta-9-THC reduced the potency of a treatment drug as an inhibitor of the twitch response. None of the delta-9-THC-induced rightward shifts in the concentration-response curves of the treatment drugs deviated significantly from parallelism. Potency ratios, confidence limits and deviations of pairs of log dose-response curves from parallelism have been determined from symmetrical (2 + 2) dose parallel line assays and were calculated from data obtained with the treatment concentrations shown (Colquhoun, 1971).

pretreated animals. For example, mean twitch amplitudes measured immediately before drug administration were 0.33 ± 0.04 g in tissues obtained from mice pretreated twice with delta-9-THC at a dose 20 mg kg$^{-1}$ and 0.28 ± 0.05 g in tissue obtained from animals pretreated twice with Tween 80 at a dose of 40 mg kg$^{-1}$ ($n = 12$). The ability of delta-9-THC to depress the twitch response was similar in vasa deferentia taken from Tween 80 pretreated mice to that in tissue taken from animals that had received no pretreatment at all (Table 3).

### *Cross-tolerance between delta-9-THC and CP 55,940, WIN 55,212-2 and anandamide*

CP 55,940, WIN 55,212-2 and anandamide shared the ability of delta-9-THC to produce a dose-dependent decrease in rectal temperature (Figure 2) and a concentration-dependent inhibition of the twitch response of the vas deferens (Figure 5). Pretreatment with two intraperitoneal injections of delta-9-THC at a dose of 20 mg kg$^{-1}$ gave rise to significant parallel rightward shifts both in the log dose-hypothermic response curves of CP 55,940 and WIN 55,212-2 (Figure 3 and Table 1) and in the log concentration-response curves of these drugs for inhibition of the twitch response (Figure 6 and Table 3). The same pretreatment with delta-9-THC also significantly attenuated the ability of anandamide to inhibit the twitch response (Figure 6 and Table 3). However, this delta-9-THC pretreatment did not produce any detectable tolerance to anandamide-induced hypothermia (Figure 3 and Table 1).

## Discussion

The results obtained in this investigation confirm previous reports that delta-9-THC, CP 55,940, WIN 55,212-2 and anandamide can produce marked decreases in mouse deep body temperature (Pertwee, 1985; Little *et al.*, 1988; Martin *et al.*, 1991; Fride & Mechoulam, 1993). In addition, the present results confirm that delta-9-THC, CP 55,940 and WIN 55,212-2 can induce a concentration-related inhibition of the electrically-evoked twitch response of the mouse vas deferens (Pacheco *et al.*, 1991; Pertwee *et al.*, 1992a,b; 1993). The ability of anandamide to inhibit the twitch response has also been reported previously (Devane *et al.*, 1992). However the earlier experiments were carried out with material extracted from pig brain whereas the samples of anandamide used in the present experiments were synthetic. The concentration-response curve of the synthetic compound (Figure 3) has proved to be essentially the same as that of the naturally occurring material, a finding that provides additional support for the conclusion drawn by Devane *et al.* (1992) that the substance extracted from the brain was indeed anandamide. The potencies of the drugs used in the present experiments exhibited the same rank order for inhibition of the twitch response of the vas deferens (Figure 5) as for the production both of hypothermia (Figure 2) and of certain other cannabimimetic effects *in vivo* (Martin *et al.*, 1991; Fride & Mechoulam, 1993): CP 55,940 > WIN 55,212-2 > delta-9-THC > anandamide. Consequently, the present data provide further support for the idea that the mouse isolated vas deferens is an appropriate preparation for investigating the central pharmacology of cannabinoids (Pertwee, 1993). It

**Table 3** Effect of *in vivo* pretreatment with delta-9-tetrahydrocannabinol (delta-9-THC) on the potencies of certain cannabimimetic drugs as inhibitors of the electrically-evoked twitch response of the mouse isolated vas deferens

| *Pretreatment drug* | *Pretreatment dose* (mg kg$^{-1}$, i.p.) | *Treatment drug* | *Treatment concentrations* (nM) | *Potency ratio* | *95% Confidence limits* | n |
|---|---|---|---|---|---|---|
| Delta-9-THC | 2 | Delta-9-THC | 10, 100 | 2.62 | 1.11 and 5.86 | 8 |
| Tween | 40 | Delta-9-THC | 3.16, 31.6 | | | |
| Delta-9-THC | 5 | Delta-9-THC | 31.6, 316 | 7.42 | 2.17 and 21.88 | 8 |
| Tween | 40 | Delta-9-THC | 3.16, 31.6 | | | |
| Delta-9-THC | 10 | Delta-9-THC | 31.6, 100 | 13.35 | 5.32 and 55.78 | 8 |
| Tween | 40 | Delta-9-THC | 3.16, 10 | | | |
| Delta-9-THC | 20 | Delta-9-THC | 31.6, 100 | 30.01 | 17.46 and 83.30 | 12 |
| Tween | 40 | Delta-9-THC | 3.16, 10 | | | |
| Delta-9-THC | 20 | CP 55,940 | 3.16, 31.6 | 8.74 | 4.30 and 17.33 | 6 |
| Tween | 40 | CP 55,940 | 0.316, 3.16 | | | |
| Delta-9-THC | 20 | WIN 55,212-2 | 3.16, 31.6 | 9.62 | 5.78 and 15.95 | 10 |
| Tween | 40 | WIN 55,212-2 | 0.316, 3.16 | | | |
| Delta-9-THC | 20 | Anandamide | 100, 1000 | 12.3 | 6.38 and 24.59 | 6 |
| Tween | 40 | Anandamide | 10, 100 | | | |
| | | | | 0.94 | 0.41 and 2.15 | 8 |
| None | – | Delta-9-THC | 3.16, 31.6 | | | |
| Tween | 40 | Delta-9-THC | 3.16, 31.6 | | | |

Mice were either unpretreated or pretreated intraperitoneally, once daily for 2 days, with delta-9-THC or with Tween 80. In the experiments in which mice were pretreated, the effects of treatment drugs, delta-9-THC, CP 55,940, WIN 55,212-2 and anandamide, on the twitch response of the vas deferens were measured measured 24 h after the second intraperitoneal injection. The final potency ratio listed in the Table indicates the extent by which the potency of delta-9-THC as an inhibitor of the twitch response was greater or less in tissues from unpretreated animals than in tissues from animals that had been pretreated with Tween 80. Pretreatment with Tween 80 did not lead to any significant change in the slope of the concentration-response curve of delta-9-THC. Each of the other potency ratios listed above indicates the extent by which pretreatment with delta-9-THC reduced the potency of a treatment drug as an inhibitor of the twitch response. None of the delta-9-THC-induced rightward shifts in the concentration-response curves of the treatment drugs deviated significantly from parallelism. Potency ratios, confidence limits and deviations of pairs of log dose-response curves from parallelism have been determined from symmetrical (2 + 2) dose parallel line assays and were calculated from data obtained with the treatment concentrations shown (Colquhoun, 1971).

should be noted that the potency of anandamide *in vivo* has previously only been compared in the same laboratory with that of delta-8-THC (Fride & Mechoulam, 1993). However, since delta-8-THC is known to be less potent than delta-9-THC as a psychotropic agent (Compton *et al.*, 1991), the finding by Fride & Mechoulam (1993) that anandamide is less potent than delta-8-THC constitutes evidence that it is also less potent than delta-9-THC.

The present results indicate that significant tolerance to the hypothermic effect of delta-9-THC can develop within 24 h of a single intraperitoneal injection of this drug. This rate of onset of tolerance to delta-9-THC is even greater than has been reported previously for the development of tolerance not only to delta-9-THC-induced hypothermia (Fitton & Pertwee, 1982) but also to other effects produced by this drug in mammalian species *in vivo* (Pertwee, 1991). It was found that tolerance could be induced both by pretreating mice with doses of delta-9-THC (10 and 20 mg kg$^{-1}$, i.p.) that are known to be hypothermic and by pretreating them with a dose (5 mg kg$^{-1}$, i.p.) that is essentially non-hypothermic at normal room temperature (Gray *et al.*, 1987). It was also found that tolerance to the hypothermic effect of delta-9-THC still develops when significant drug-induced decreases in deep body temperature are prevented during the pretreatment period by raising the ambient temperature. It seems unlikely, therefore, that mice need to experience delta-9-THC-induced hypothermia in order to become tolerant to this effect.

Tolerance to the inhibitory effect of delta-9-THC on the electrically-evoked twitch response of the mouse vas deferens was found to develop no less rapidly than tolerance to the drug's hypothermic effect. For both effects, the degree of tolerance produced was related to the size of the pretreatment dose of delta-9-THC used and to the number of pretreatments given, supporting the idea that the extent to which the tissues of the body are exposed to cannabinoids is an important factor in the development of cannabinoid tolerance (Pertwee, 1991). The mouse vas deferens is one of only two *in vitro* preparations in which it has been found possible to induce cannabinoid tolerance by prior *in vivo* cannabinoid administration, the other being the myenteric plexus-longitudinal muscle preparation of the mouse small intestine (Pertwee *et al.*, 1992b). The finding that the sensitivity of the mouse vas deferens to delta-9-THC can be attenuated by *in vivo* pretreatment with delta-9-THC suggests that this tissue is suitable as a model with which to explore the basis of cannabinoid tolerance. This idea is reinforced by the observation in the present investigation that *in vivo* pretreatment with delta-9-THC can produce tolerance not only to its own inhibitory effect on the twitch response of the vas deferens but also to that of three other drugs, CP 55,940, WIN 55,212-2 and anandamide (Figure 6), each of which is known to bind avidly to cannabinoid binding sites and to possess cannabimimetic pharmacological properties *in vivo* (Little *et al.*, 1988; Martin *et al.*, 1991; Pacheco *et al.*, 1991; Devane *et al.*, 1992; Howlett *et al.*, 1992; Jansen *et al.*, 1992; Fride & Mechoulam, 1993; Pertwee, 1993).

The demonstration that an isolated tissue can be made tolerant to psychotropic cannabinoids strengthens the hypothesis that cannabinoid tolerance is primarily pharmacodynamic in nature rather than dispositional or metabolic (Pertwee, 1991). The observation that cross tolerance can develop between delta-9-THC and CP 55,940, WIN 55,212-2 and anandamide in the vas deferens may constitute further evidence for this hypothesis if, as seems likely, these chemically quite unrelated compounds are not all inactivated by the same

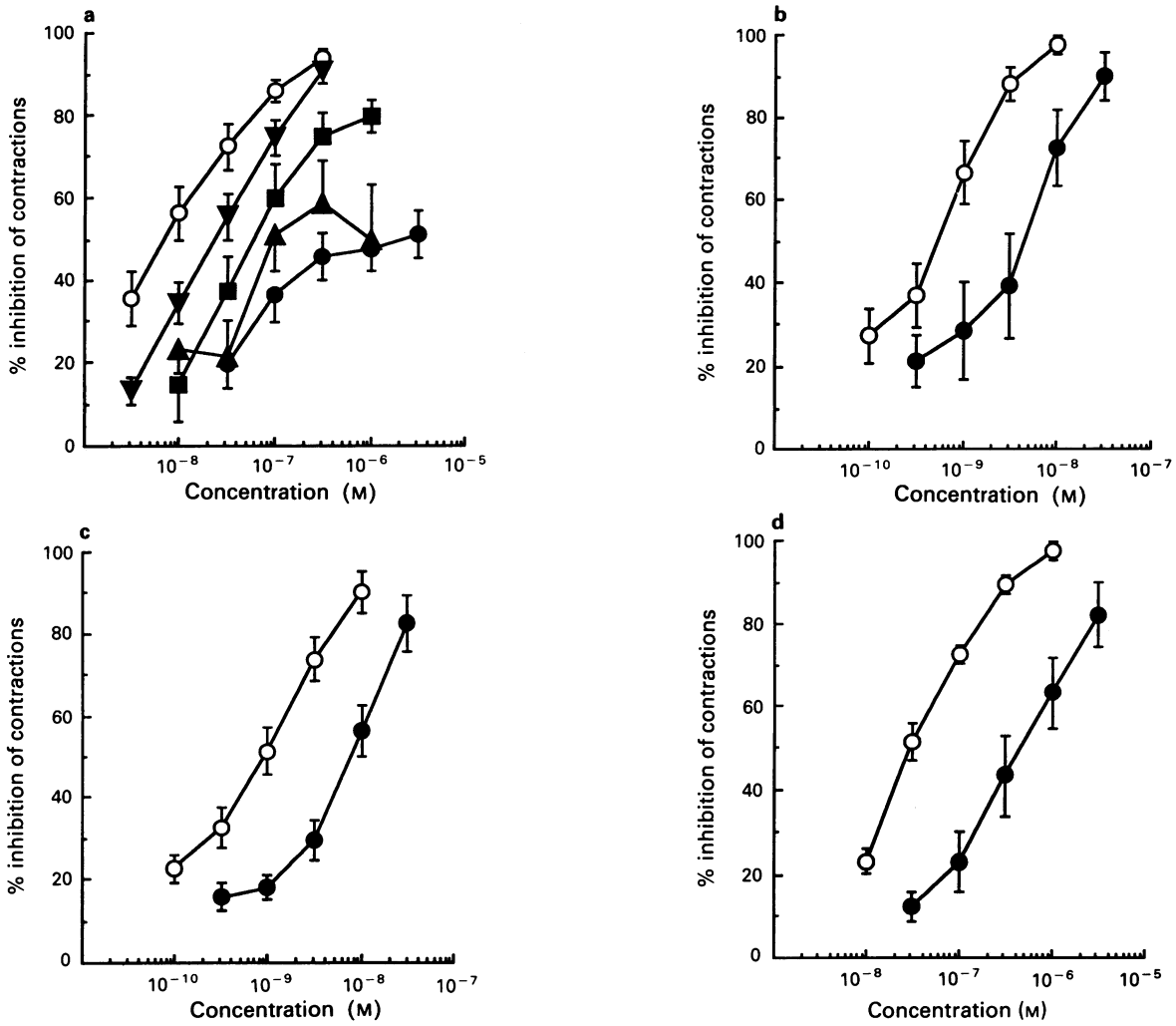


**Figure 6** Mean cumulative concentration-response curves for (a) delta-9-tetrahydrocannabinol (delta-9-THC), (b) CP 55,940, (c) WIN 55,212-2, and (d) anandamide in isolated vasa deferentia obtained from mice pretreated once daily for 2 days with Tween 80 at a dose of 40 mg $kg^{-1}$, i.p. (○) or with delta-9-THC at doses of 2 mg $kg^{-1}$, i.p. (▼), 5 mg $kg^{-1}$, i.p. (■), 10 mg $kg^{-1}$, i.p. (▲) or 20 mg $kg^{-1}$, i.p. (●). Each symbol represents the mean value ± s.e.mean of inhibition of electrically-evoked contractions expressed as a percentage of the amplitude of the twitch response measured immediately before the first addition of drug to the organ bath ($n = 6$ to 12 different vasa deferentia). The amplitudes of contractions evoked immediately before drug administration in tissues obtained from mice pretreated twice with delta-9-THC were not significantly different from those evoked in tissues obtained from vehicle-pretreated animals.

metabolic pathways. The same observation may also support the idea that delta-9-THC, CP 55,940, WIN 55,212-2 and anandamide share a common mode of action, the strength of this support depending on whether or not it transpires that cross tolerance also develops in the mouse vas deferens between delta-9-THC and inhibitors of the twitch response that do not possess cannabimimetic properties.

It was found that *in vivo* pretreatment with delta-9-THC induced significantly greater tolerance to its own inhibitory effect on the twitch response of the vas deferens than to that of CP 55,940 or WIN 55,212-2. It was also found that the degree of tolerance to delta-9-THC induced by such pretreatment was significantly greater for inhibition of the twitch response than for the production of hypothermia. The reasons for these differences remain to be established. It is worth noting, however, that there are already reports in the literature that tolerance develops more readily to some effects of cannabinoids than to others (Pertwee, 1991). Whilst the pretreatment with delta-9-THC that produced tolerance to the effects of delta-9-THC, CP 55,940 and WIN 55,212-2 on body temperature and on the twitch response of the vas deferens also produced tolerance to anandamide-induced inhibition of the twitch response, it did not produce any detectable tolerance to the effect of anandamide on body temperature. Given the hypothesis that anandamide is an endogenous cannabinoid it is important that the explanation for this apparent anomaly is found. One possibility is that the mode of action of anandamide for the production of hypothermia is different from that of the other cannabimimetic agents used in the present investigation, the compound perhaps achieving its hypothermic effect not only by interacting with cannabinoid receptors but also by acting through less specific mechanisms that do not develop tolerance to cannabimimetic agents. Consistent with this hypothesis are the present findings that the effective hypothermic dose-range of anandamide is rather high and that the log dose-hypothermic response curve of anandamide is somewhat shallower than that of delta-9-THC, CP 55,940 or WIN 55,212-2 (Figure 1).

We thank the Wellcome Trust for financial support, the National Institute on Drug Abuse, U.S.A., for delta-9-THC, Dr L.S. Melvin (Pfizer) for CP 55,940, Dr S.J. Ward (Sterling Winthrop) for WIN 55,212-2 and Professor R. Mechoulam (University of Jerusalem) for anandamide.

## References


COLQUHOUN, D. (1971). *Lectures on Biostatistics*. Oxford: Oxford University Press.

COMPTON, D.R., PRESCOTT, W.R., MARTIN, B.R., SIEGEL, C., GORDON, P.M. & RAZDAN, R.K. (1991). Synthesis and pharmacological evaluation of ether and related analogues of delta-8, delta-9, and delta-9,11-tetrahydrocannabinol. *J. Med. Chem.*, **34**, 3310–3316.

DEVANE, W.A., HANUS, L., BREUER, A., PERTWEE, R.G., STEVENSON, L.A., GRIFFIN, G., GIBSON, D., MANDELBAUM, A., ETINGER, A. & MECHOULAM, R. (1992). Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science*, **258**, 1946–1949.

DUNNETT, C.W. (1964). New tables for multiple comparisons with a control. *Biometrics*, **20**, 482–491.

FITTON, A.G. & PERTWEE, R.G. (1982). Changes in body temperature and oxygen consumption rate of conscious mice produced by intrahypothalamic and intracerebroventricular injections of delta-9-tetrahydrocannabinol. *Br. J. Pharmacol.*, **75**, 409–414.

FRIDE, E. & MECHOULAM, R. (1993). Pharmacological activity of the cannabinoid receptor agonist, anandamide, a brain constituent. *Eur. J. Pharmacol.*, **231**, 313–314.

GRAY, G.A., HEDLEY, D. & PERTWEE, R.G. (1987). Enhancement of the hypothermic response of mice to delta-9-tetrahydrocannabinol by subhypothermic doses of chlorpromazine and phentolamine. *Neuropharmacology*, **26**, 229–235.

GRIFFIN, G. & PERTWEE, R.G. (1993). Vasa deferentia obtained from mice pretreated with delta-9-tetrahydrocannabinol (THC) show tolerance to delta-9-THC, WIN 55,212-2 and CP 55,490. *Br. J. Pharmacol.*, **109**, 105P.

HOWLETT, A.C., EVANS, D.M. & HOUSTON, D.B. (1992). The cannabinoid receptor. In *Marijuana/Cannabinoids. Neurobiology and Neurophysiology*. ed. Murphy, L. & Bartke, A. pp. 35–72. Boca Raton: CRC Press.

JANSEN, E.M., HAYCOCK, D.A., WARD, S.J. & SEYBOLD, V.S. (1992). Distribution of cannabinoid receptors in rat brain determined with aminoalkylindoles. *Brain Research*, **575**, 93–102.

LITTLE, P.J., COMPTON, D.R., JOHNSON, M.R., MELVIN, L.S. & MARTIN, B.R. (1988). Pharmacology and stereoselectivity of structurally novel cannabinoids in mice. *J. Pharmacol. Exp. Ther.*, **247**, 1046–1051.

MARTIN, B.R., COMPTON, D.R., THOMAS, B.F., PRESCOTT, W.R., LITTLE, P.J., RAZDAN, R.K., JOHNSON, M.R., MELVIN, L.S., MECHOULAM, R. & WARD, S.J. (1991). Behavioural, biochemical, and molecular modeling evaluations of cannabinoid analogs. *Pharmacol. Biochem. Behav.*, **40**, 471–478.

PACHECO, M., CHILDERS, S.R., ARNOLD, R., CASIANO, F. & WARD, S.J. (1991). Aminoalkylindoles: actions on specific G-protein-linked receptors. *J. Pharmacol. Exp. Ther.*, **257**, 170–183.

PERTWEE, R.G. (1985). Effects of cannabinoids on thermoregulation: a brief review. In *Marihuana '84*. ed. Harvey, D.J. pp. 263–277. Oxford: IRL Press.

PERTWEE, R.G. (1991). Tolerance to and dependence on psychotropic cannabinoids. In *The Biological Bases of Drug Tolerance and Dependence*. ed. Pratt, J.A. pp. 231–263. London: Academic Press.

PERTWEE, R.G. (1993). The evidence for the existence of cannabinoid receptors. *Gen. Pharmacol.*, **24**, 811–824.

PERTWEE, R.G., STEVENSON, L.A., ELRICK, D.B., MECHOULAM, R. & CORBETT, A.D. (1992a). 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**, 980–984.

PERTWEE, R.G., STEVENSON, L.A., FERNANDO, S.R. & CORBETT, A.D. (1992b). The production of cannabinoid tolerance in preparations of mouse vas deferens and of myenteric plexus-longitudinal muscle of mouse small intestine. *Br. J. Pharmacol.*, **107**, 365P.

PERTWEE, R.G., STEVENSON, L.A., FERNANDO, S.R. & CORBETT, A.D. (1993). *In vitro* effects of the cannabinoid, CP 55,940, and of its (+)-enantiomer, CP 56,667. In *Problems of Drug Dependence, 1992: Proceeding of the 54th Annual Scientific Meeting of the College on Problems of Drug Dependence, Inc.: National Institute on Drug Abuse Research Monograph Series, Research Monograph*, 132. ed. Harris, L. p. 374. Rockville: National Institute on Drug Abuse.

PERTWEE, R.G. & TAVENDALE, R. (1977). Effects of delta-9-tetrahydrocannabinol on the rates of oxygen consumption of mice. *Br. J. Pharmacol.*, **60**, 559–568.


*(Received August 4, 1993)*
*Accepted September 1, 1993)*