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Development of Cross-Tolerance between Δ^9-Tetrahydrocannabinol, CP 55,940 and WIN 55,212[1]

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ABSTRACT

In previous studies it was shown that the structurally dissimilar compounds Δ^9-THC, CP 55,940 and WIN 55,212 produced more or less the same pharmacological effects and interacted with the same cannabinoid receptor. However, their potencies vary across a number of pharmacological assays, suggesting that a single mechanism may not account for all of their actions. To further explore possible differences among these cannabinoids, cross-tolerance studies were conducted. Specifically, the ability of Δ^9-THC, CP 55,940 and WIN 55,212 to produce hypoactivity, hypothermia, antinociception and catalepsy was assessed in mice that had been chronically treated with either Δ^9-THC or CP 55,940. The results indicated the Δ^9-THC-treated mice were tolerant to Δ^9-THC. The degrees of tolerance were 15.9, 7.8, and 13.4 for spontaneous activity, hypothermia and antinociception, respectively. Mice chronically treated with Δ^9-THC also exhibited tolerance to some of the behavioral effects of CP 55,940 and WIN 55,212. The tolerance induced by repetitive administration of CP 55,940 was substantial. The ED_{50} for CP 55,940 was shifted 102 fold for spontaneous activity, 100 for hypothermia and 44 for catalepsy. Also, some cross-tolerance to Δ^9-THC and WIN 55,212 was observed in CP 55,940 chronically treated mice. These findings indicate that cross-tolerance develops between Δ^9-THC, CP 55,940 and WIN 55,212 and that these agents have some actions in common. However, quantitative differences in their development of cross-tolerance suggests that all of their actions may not be identical.

Δ^9-THC, a component in marijuana, produces characteristic psychotropic responses in humans as well as specific behavioral alterations in laboratory animals such as depression of motor activity, hypothermia, antinociception, static ataxia, catalepsy, hyperexcitability, anticonvulsant activity and the ability to produce a discriminative stimulus cue (Dewey, 1986). Early structure-activity relationship studies provided a compelling argument for a cannabinoid receptor (Razdan, 1986). However, these studies more or less relied on the traditional three-ringed benzopyran structure of Δ^9-THC (fig. 1). The introduction of bicyclic analogs with extended branched side chains, such as CP 55,940 depicted in figure 1, reinforced the receptor concept with their enhanced potency and revolutionized the structure-activity relationship concept for cannabinoids. Moreover, ^{3}H-CP 55,940 was used to characterize cannabinoid receptor binding (Devane *et al.*, 1988). Although CP 55,940 represented a certain degree of structural diversity, the discovery of cannabinoid activity in aminoalkylindoles demonstrated that Δ^9-THC-like effects could be produced by a structurally distinct class of compounds (Pacheco *et al.*, 1991; Compton *et al.*, 1992a). In addition to their pharmacological similarities, these three compounds also inhibited adenylyl cyclase (Howlett and Fleming, 1984; Pacheco *et al.*, 1991) and appeared to act at the same receptor (Devane *et al.*, 1988; Compton *et al.*, 1993; Pacheco *et al.*, 1993). Additionally, a molecular modeling study revealed that there is a pharmacophore which appears to accommodate all of these compounds (Thomas *et al.*, 1991). These findings strongly suggest that at least some of the pharmacological effects of Δ^9-THC, CP 55,940 and WIN 55,212 are mediated through a specific cannabinoid receptor.

The data from our laboratory concerning the behavioral potencies of Δ^9-THC, CP 55,940 and WIN 55,212 with regard to production of hypoactivity, hypothermia, antinociception and catalepsy in mice demonstrated that these agents produced more or less the same pharmacological effects, which supports the conclusion that they act in a similar fashion (Abood and Martin, 1992; Compton *et al.*, 1992a,b). However, these data also suggest some possible differences between Δ^9-THC, CP 55,940 and WIN 55,212. Δ^9-THC was almost equipotent in producing these four effects, whereas CP 55,940 and WIN 55,212 exhibited differing potencies in the various measurements. For example, CP 55,940 was almost 10 times more potent in reducing motor activity than in

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ABBREVIATIONS: THC, tetrahydrocannabinol; ED_{50}, 50% effective dose; %MPE, percent maximum possible effect.

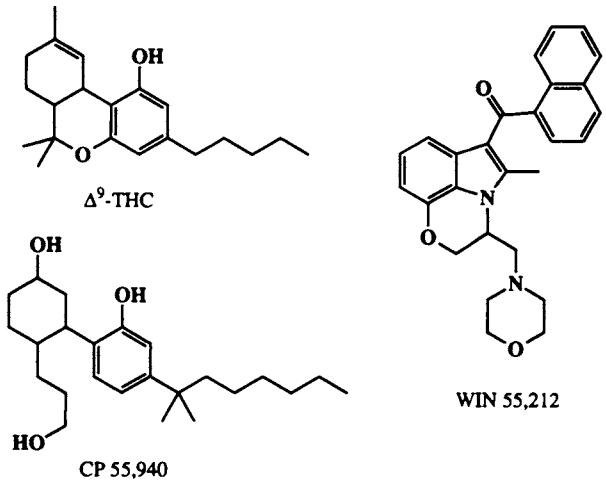

Fig. 1. Structures of Δ^9-THC, CP 55,940 and WIN 55,212.

producing catalepsy. WIN 55,212 was 12 times more potent in producing hypoactivity than in reducing rectal temperature. Additionally, the maximum effects of these compounds on each behavior were not identical. These differences raise the possibility that these agents may activate multiple biochemical processes rather than a single receptor-effector system.

Since the *in vivo* and *in vitro* models described above may not be sufficient to distinguish differences in pharmacological and biochemical effects of Δ^9-THC, CP 55,940 and WIN 55,212 if they do exist, cross-tolerance studies were conducted with each drug in animals chronically treated with Δ^9-THC or CP 55,940. Additionally, development of cross-tolerance among these agents would further validate the use of WIN 55,212–2 and CP 55,940 as cannabinoid probes.

Materials and Methods

ICR male mice (Harlan Laboratories, Indianapolis, IN) weighing 24 to 26 g were used in all experiments. Mice were maintained on a 14:10-hr light/dark cycle with free access to food and water. Δ^9-THC was obtained from the National Institute on Drug Abuse (Bethesda, MD). CP 55,940 and WIN 55,212 were provided by Pfizer (Groton, CT) and Sterling Drug Inc. (Rensselaer, NY), respectively.

Drug preparation and chronic drug treatment. Cannabinoids were dissolved in a 1:1:18 mixture of ethanol, emulphor and saline. For Δ^9-THC tolerance, mice were injected s.c. twice daily with either Δ^9-THC (10 mg/kg) or vehicle for 6 days. On day 7, the mice received only a single s.c. injection of Δ^9-THC and 24 hr later an i.v. injection of the appropriate test drug. For CP 55,940 tolerance, mice were given s.c. injections twice daily with either CP 55,940 (2 mg/kg) or saline for 13 days. On day 14, the mice received a single injection of the test drug in the morning and 24 hr later an i.v. injection of the appropriate test drug. Six groups of animals treated with vehicle or drug (six mice per group) were evaluated in a single experiment, which included a vehicle group as the control. Dose-response relationships for each cannabinoid were determined in both vehicle or drug-treated groups. Data were expressed as the combination of at least two independent experiments with at least 12 animals per group.

Pharmacological assays. All animals were acclimated to the observation room overnight. Twenty-four hours after the last injection of either Δ^9-THC or CP 55,940, animals in both drug- and vehicle-treated groups received the test agent by tail-vein injection and evaluated for their ability to produce hypomotility, hypothermia, antinociception and catalepsy. These four pharmacological measures were determined in the same mouse at a time when maximal activity was present (Little *et al.*, 1989). To measure locomotor activity, mice were placed into individual photocell activity chambers (11 inches × 6.5 inches) 5 min after injection. Spontaneous activity was measured during the next 10-min period as the number of interruptions of 16 photocell beams per chamber. Antinociception was determined using the tail-flick reaction time to a heat stimulus (Dewey *et al.*, 1970). Before vehicle or drug administration, the baseline latency period (2–3 sec) was determined. Twenty minutes after the injection, tail-flick latency was assessed once more, and the difference in control and test latencies were calculated. A 10-sec maximum latency was used. Antinociception was expressed as % MPE as described below. As for hypothermia, rectal temperature was determined prior to vehicle or drug administration with a telethermometer (Yellow Springs Instrument CO., Yellow Springs, OH) and a thermistor probe (model YSI 400, Markson, Inc.) inserted at a depth of 2 mm. At 60 min post-injection, rectal temperature was measured again, and the difference between pre- and post-injection values was calculated. Catalepsy, or ring-immobility, was determined by using a slight modification of the ring test (Pertwee, 1972). Each mouse was placed on a ring (5.5 cm in diameter) that was attached to a ring stand and raised to a height of 16 cm. Mice were allowed to stay for a period of 5 min during which the sum of all times the mouse was motionless (except for respiratory movements) was measured to the nearest second. Mice that either fell or jumped from the ring were allowed five such "escapes." If these occurred before 2.5 min, the animal was eliminated from the study. Catalepsy was measured at 90 min after treatment.

Data analysis. For production of hypomotility and hypothermia, the data were expressed as percent of control activity and change in °C, respectively. Antinociception was calculated as % MPE = [test latency − control latency)/(10 sec − control latency)] × 100. Catalepsy was determined as: % Immobility = [(amount of time immobile)/(length of test session)] × 100. Six groups of six animals per group were treated chronically with either vehicle or drug. An acute vehicle group was included as a control on the day of testing. Dose-response relationships for each cannabinoid were determined in both vehicle- and drug-treated groups. Data are expressed as the combination of at least two independent experiments. The data from both vehicle- and drug-treated groups were evaluated by the Allfit program (De Lean *et al.*, 1978) to determine the ED_{50} value, to evaluate the parallel shifts of the dose-response curves, and to assess significance of the data ($P < .05$).

Results

Induction of tolerance to Δ^9-THC. To conduct cross-tolerance studies, a preliminary investigation indicated that at least 1 week of treatment with twice daily doses of Δ^9-THC (10 mg/kg) were required to produce reliable tolerance. Studies were then conducted by treating mice twice daily with vehicle or 10 mg/kg Δ^9-THC for either 6 or 13 days plus a single injection on the last treatment day (day 7 and 14, respectively). Dose-response curves for Δ^9-THC were generated the following morning after the last day of treatment. The results, depicted in figure 2 and summarized in table 1, show that the two treatment regimens produced comparable tolerance in the tail-flick and immobility assays. There appeared to be some slight difference between the 6- and 13-day regimens for development of tolerance in the spontaneous activity and hypothermia assays. The tolerance was clearly no greater in the 13-day treated animals and in fact appears to be somewhat less for spontaneous activity and rectal temperature. For these reasons, the 6-day treatment regimen was chosen for the cross-tolerance studies. In this regimen, the degree of tolerance to Δ^9-THC was determined to be 15.9,

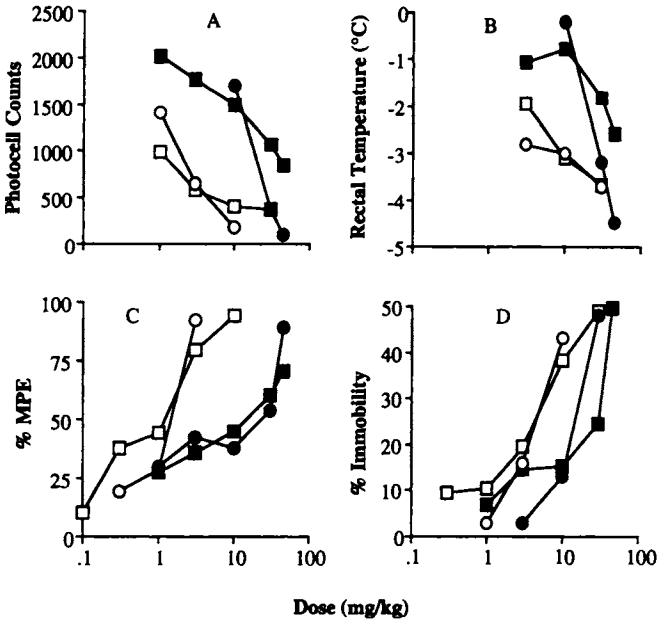

Fig. 2. Dose-response relationship of i.v. administered Δ^9-THC on spontaneous activity (A), rectal temperature (B), antinociception (C) and catalepsy (D) in mice treated 6 days with either vehicle (□) or Δ^9-THC (■) or 13 days with either vehicle (○) or Δ^9-THC (●). The chronic administration consisted of twice daily s.c. injections of Δ^9-THC (10 mg/kg).

13.4, 7.8 and 4.4 fold for spontaneous activity, antinociception, hypothermia and catalepsy, respectively. The shifts in dose-response curves were parallel and statistically significant, with the exception of catalepsy, which was not statistically significant. However, the degree of tolerance which developed to the cataleptic effect of Δ^9-THC with the 6- and 13-day treatment regimens appeared to be comparable, and the 13-day treatment regimen was statistically significant, suggesting tolerance, though minimal for catalepsy, did develop.

CP 55,940 and WIN 55,212 cross-tolerance to Δ^9-THC. Mice subjected to the 6-day treatment with Δ^9-THC also exhibited statistically significant tolerance to the effects of CP 55,940 on spontaneous activity (3.6 times) and antinociception (3.6 times) as given in table 1 and shown in figure 3. The dose-response curve for CP 55,940 was shifted 1.9 times for hypothermia and 1.6 times for catalepsy, but these shifts were not statistically significant (table 1). Similarly, Δ^9-THC treated mice were also tolerant to the effects of WIN 55,212 with regard to hypothermia (3.2 times), antinociception (2.9 times) and immobility (1.6 times) even though the tolerance to the latter was modest (fig. 4). Oddly, the tolerance (2.6 fold) that developed to the locomotor effects was not statistically significant. The dose-response curves were found to be parallel when there were statistically significant shifts.

Induction of tolerance to CP 55,940. The initial studies that were carried out to establish parameters for producing tolerance to CP 55,940 are presented in table 2. Mice were treated twice daily with either vehicle (0 mg/kg), 2 or 4 mg/kg CP 55,940 for 6 days, given a single injection s.c. the last day and then evaluated on the following morning with an acute i.v. test dose (0.3 mg/kg) of CP 55,940. CP 55,940 produced potent effects in all four behavioral assays in the vehicle-treated mice, whereas it was completely ineffective in altering spontaneous activity, body temperature and catalepsy in the CP 55,940-treated animals. Although there appeared to be some attenuation in the CP 55,940 antinociceptive effects in both 2 and 4 mg/kg treated groups, there was a lack of significant tolerance development. Therefore, the treatment regimen was extended to 13 days plus one (27 injections) and a higher test dose (1.0 mg/kg) of CP 55,940 was evaluated for possible tolerance. The results in table 2 again show that complete tolerance developed to all effects except antinociception. However, the failure of CP 55,940 to produce 100% antinociception at a dose of 1.0 mg/kg in CP 55,940-treated mice suggested that some tolerance had developed, considering the ED_{50} for CP 55,940 is 0.09 mg/kg in naive mice (Compton *et al.*, 1992b). Complete dose-response curves for CP 55,940 were generated in mice treated for 6 and 13 days with either vehicle or CP 55,940 (2 mg/kg), and the results are shown in figure 5 and summarized in table 3. Similar results were obtained using both treatment regimens in that substantial tolerance developed to the hypoactivity, hypothermia and catalepsy induced by CP 55,940. The degrees of tolerance in the 13-day treated mice were 102, 100, and 44 times for spontaneous activity, rectal temperature and immobility, respectively. Statistical analysis of these data revealed that the shifts were parallel and significant. In the antinociceptive assay, the effect of CP 55,940 in the 6-day

TABLE 1

Effects of i.v. administered Δ^9-THC, CP 55,940 and WIN 55,212 on spontaneous activity, rectal temperature, antinociception and catalepsy in mice treated s.c. with Δ^9-THC (10 mg/kg twice daily for 6 days)

			ED_{50} (mg/kg)			
Chronic Treatment	Days	Test	Hypoactivity	Hypothermia	Antinociception	Catalepsy
Vehicle	6	Δ^9-THC	1.5	4.6	0.8	3.9
Δ^9-THC	6	Δ^9-THC	23.8	36.0	10.7	17.3
Potency ratio			15.9*	7.8*	13.4*	4.4
Vehicle	13	Δ^9-THC	2.5	2.6	1.2	3.7
Δ^9-THC	13	Δ^9-THC	13.3	21.7	9.5	13.1
Potency ratio			5.3*	8.3*	7.9	3.5*
Vehicle	6	CP 55,940	0.037	0.140	0.045	0.208
Δ^9-THC	6	CP 55,940	0.135	0.265	0.162	0.326
Potency ratio			3.6*	1.9	3.6*	1.6
Vehicle	6	WIN 55,212	1.09	3.01	0.496	6.79
Δ^9-THC	6	WIN 55,212	2.84	9.78	1.42	10.9
Potency ratio			2.6	3.2*	2.9*	1.6*

* ED_{50} was significantly different between the vehicle- and drug-tested groups at $P < .05$, and the shift was parallel. The ED_{50}'s were determined from dose-response curves constructed with a minimum of three doses and with at least 12 mice per dose.

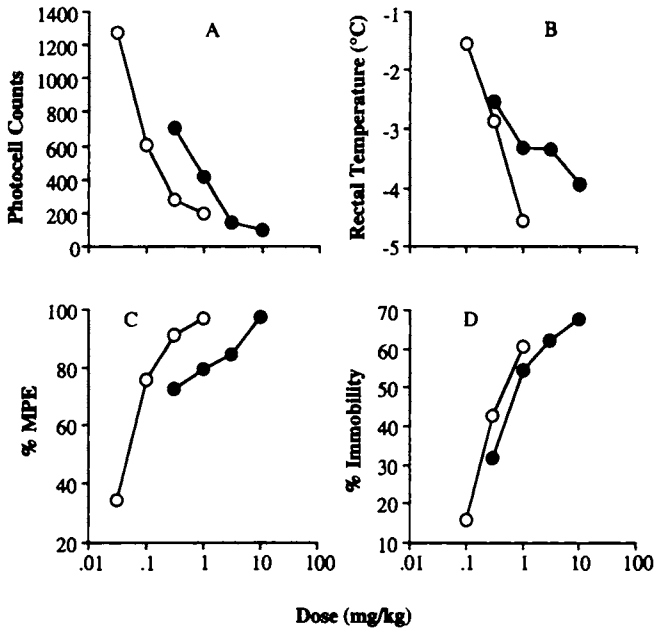

Fig. 3. Dose-response relationship of i.v. administered CP 55,940 on spontaneous activity (A), rectal temperature (B), antinociception (C) and catalepsy (D) in mice treated s.c. with either vehicle (○) or 10 mg/kg Δ^9-THC (●) for 6 days.

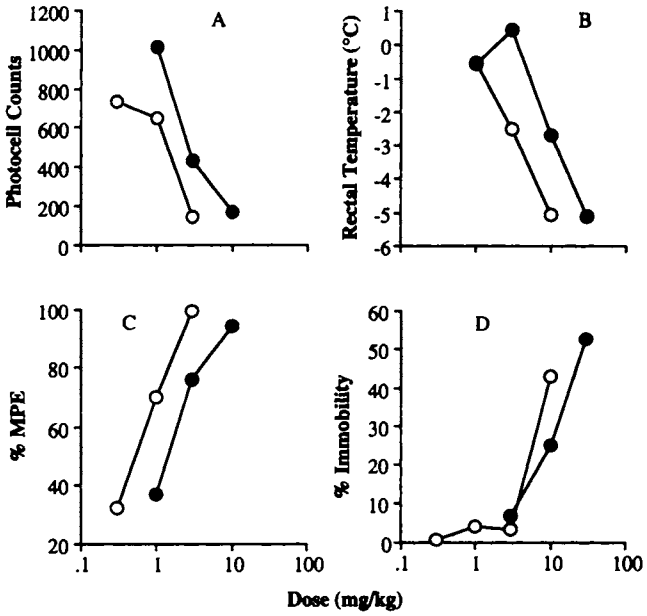

Fig. 4. Dose-response relationship of i.v. administered WIN 55,212 on spontaneous activity (A), rectal temperature (B), antinociception (C) and catalepsy (D) in mice treated s.c. with either vehicle (○) or 10 mg/kg Δ^9-THC (●) for 6 days.

treated mice was not dose related (fig. 5) and therefore an ED_{50} could not be calculated. Although it is difficult to interpret these data, it does appear that little tolerance developed to the lower doses of CP 55,940 whereas a high degree of tolerance appears to have developed at high doses. For example, a dose of 0.3 mg/kg of CP 55,940 in vehicle-treated mice produced a response that was comparable to that produced by a dose of 60 mg/kg in CP 55,940-treated mice. Clearly, the lack of a dose-related response is atypical of cannabinoid-induced antinociception.

Δ^9-THC and WIN 55,212 cross-tolerance to CP 55,940. Cross-tolerance studies were carried out using the 13-day regimen because greater tolerance developed to CP 55,940-induced catalepsy with this regimen. Also, in light of the difficulty in demonstrating dose-related tolerance to the CP 55,940 antinociceptive effects, efforts were made to maximize the possibility of producing antinociceptive cross-tolerance by using the 13-day schedule. The results of the effects of acute Δ^9-THC (fig. 6) and WIN 55,212 (fig. 7) in CP 55,940-tolerant mice indicated that cross-tolerance developed (table 3). The degree of cross-tolerance to the effects Δ^9-THC were determined to be 6.0 fold for spontaneous activity, 4.8 for hypothermia, 15.5 for antinociception and 6.5 for catalepsy, respectively. Similarly, it was found that CP 55,940-tolerant mice were cross-tolerant to WIN 55,212-induced hypoactivity (11.3), hypothermia (82.3), antinociception (18.8) and catalepsy (15.0). Interestingly, statistical analysis indicated that the shift of the dose-response curve for the Δ^9-THC effect on spontaneous activity was not parallel, and due to limited data no quantification of the shift could be made, though tolerance obviously developed to the inhibitory effect of Δ^9-THC on locomotor activity (fig. 6) as indicated by the fact that about 50% inhibition occurred at a dose of 3 mg/kg in vehicle-treated mice whereas doses up to 20 mg/kg produced no apparent effect in CP 55,940-treated mice. Additionally, the dose-response curves for WIN 55,212-induced hypoactivity

TABLE 2

Influence of dose and treatment time on the development of tolerance to CP 55,940

Animals received s.c. injections of the indicated doses (mg/kg) of CP 55,940 twice a day for the indicated number of days. The animals received an i.v. injection of the test dose of CP 55,940 on the following morning after the last chronic treatment. Results are presented as means ± SEM (n = 6).

Chronic Treatment		Test Dose[a]	Hypoactivity[b]	Hypothermia[c]	Antinociception[d]	Catalepsy[e]
Days	Dose[a]					
6	0	0.3	192 ± 57	−4.9 ± 0.4	100 ± 0	53 ± 4
6	2	0.3	2589 ± 554	0.1 ± 0.3	68 ± 16	3 ± 1
6	4	0.3	2996 ± 578	−0.3 ± 0.5	67 ± 15	2 ± 1
13	0	1.0	346 ± 95	−4.6 ± 0.4	100 ± 0	38 ± 11
13	2	1.0	3028 ± 28	−0.3 ± 0.3	74 ± 7	10 ± 4
13	4	1.0	2314 ± 640	0.1 ± 0.3	68 ± 15	6 ± 2

[a] Doses expressed as mg/kg.
[b] Interruption of photocell counts for a 10-min period.
[c] Changes in rectal temperature (°C) from baseline.
[d] %MPE.
[e] %Immobility.

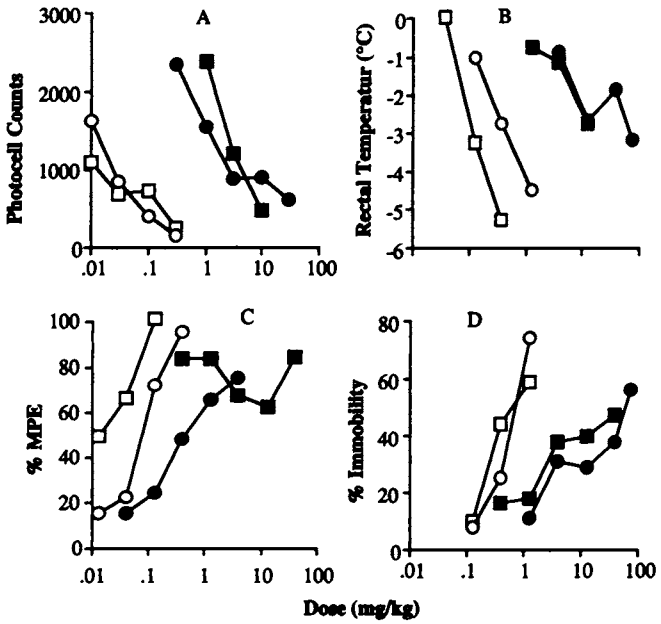

Fig. 5. Dose-response curves of i.v. administered CP 55,940 on spontaneous activity (A), rectal temperature (B), antinociception (C) and catalepsy (D) in mice treated s.c. for 6 days with either vehicle (□) or 2 mg/kg CP 55,940 (■) or 13 days with either vehicle (○) or 2 mg/kg CP 55,940 (●).

were not parallel in the vehicle- and CP 55,940-treated groups.

Discussion

The phenomenon of Δ^9-THC-induced tolerance has been well documented in pigeons, dogs, rats, mice, rabbits, hamsters, monkeys and gerbils (Pertwee, 1991). The data presented here clearly showed that chronic Δ^9-THC treatment in mice resulted in development of tolerance to Δ^9-THC-induced hypoactivity, hypothermia and antinociception, which was consistent with previous reports (Anderson *et al.*, 1974; Fitton and Pertwee, 1982; Martin, 1985). The degree of tolerance developed by Δ^9-THC in mice was moderate (8–16 fold), compared with some sensitive species such as the pigeon (100 fold). It has been shown that tolerance develops to Δ^9-THC-induced catalepsy in mice (Pertwee, 1991), but only modest tolerance was observed in our study, which may reflect differences in experimental conditions. Although an exhaustive attempt to determine the maximum possible tolerance to Δ^9-THC was not undertaken, a comparison of the 6- and 13-day treated-animals suggest that greater tolerance in mice may not be readily attained.

In the past several decades, most of the cannabinoid research has been directed mainly toward Δ^9-THC until a large number of synthetic cannabinoid analogs emerged, one of which was CP 55,940. It is approximately 20–75 times more potent than Δ^9-THC in producing behavioral effects, depending upon the pharmacological measure. However, little is known about the ability of CP 55,940 to produce tolerance. In a recent report, CP 55,940 administration to rats for 14 days resulted in tolerance development to its effects on open field behavior (Oviedo *et al.*, 1993); however, no attempts were made to quantitate the degree of tolerance. Since CP 55,940 is being used as a probe for the cannabinoid receptor, it is essential that its actions be thoroughly characterized. In the present study, it was found that dramatic tolerance developed to all CP 55,940 effects. The degrees of tolerance for spontaneous activity, rectal temperature and catalepsy ranged between 44 and 100 times for the 13-day treated animals, which were only marginally greater than those in the 6-day treated animals. In contrast to the straightforward tolerance that developed to CP 55,940-induced hypoactivity, hypothermia and catalepsy, the CP 55,940-induced antinociceptive response produced in CP 55,940-treated animals was quite complex. Attempts to demonstrate tolerance to the antinociceptive effects of CP 55,940 failed to produce a dose-related response following 6 days of treatment. A rather constant effect (approximately 60%) was observed throughout a wide range of doses (1–30 mg/kg). However, a very different response was observed in the 13-day treated mice. A wide range of CP 55,940 doses in these tolerant animals resulted in a nonlinear curve. ALLFIT analysis indicates a 2.9 times (but not statistically significant) shift. However, the CP 55,940 dose-response curve following this 13-day treatment regimen, though parallel with that obtained in the vehicle-treated group, reached a plateau at 70% MPE with doses greater that 10 mg/kg. It was impossible to statistically fit all cluster points to a single curve, and a fit could only be obtained for the portion of the curve represented by doses up to 10 mg/kg (the first point of inflection in the response curve). There is no plausible explanation for CP 55,940's antinociceptive tolerance that did not occur with either Δ^9-THC or WIN 55,212. The results with these 6- and 13-day treatment protocols, along with the preliminary data, suggested that maximum tolerance for CP 55,940 was obtained in these four pharmacological assays. Collectively, these data confirmed that tolerance develops to the effects of CP 55,940 as it does for Δ^9-THC, with the primary difference being that the magnitude of CP 55,940 tolerance is considerably greater than that produced by Δ^9-THC in all of the assays except antinociception.

Cross-tolerance studies among certain psychoactive cannabinoids have been well documented. They include natural cannabinoids (Δ^9-THC, Δ^8-THC), metabolites of cannabinoids (11-OH-Δ^9-THC, 11-OH-Δ^8-THC) and synthetic cannabinoids (*e.g.*, dimethylheptyl-THC). It appears that cross-tolerance is a general phenomenon among these behaviorally active cannabinoids (McMillan *et al.*, 1971). In the present study, mice made tolerant to Δ^9-THC were also moderately tolerant to the effects of CP 55,940 for reducing motor activity and producing antinociception. It was also found that the mice under the same chronic treatment exhibited moderate tolerance to the effect of WIN 55,212 on producing hypothermia, antinociception and catalepsy. However, cross-tolerance to CP 55,940 and WIN 55,212 in Δ^9-THC tolerant mice did not attain statistical significance for all effects. For example, mice tolerant to Δ^9-THC did not show significant tolerance to CP 55,940 on producing hypothermia and catalepsy, and to WIN 55,212 in producing hypoactivity. Recently, it was reported that cross-tolerance developed to the hypothermic effect of CP 55,940 and WIN 55,212 in Δ^9-THC-treated mice (Pertwee *et al.*, 1993). These investigators used a shorter treatment regimen (two days) with higher doses (20 mg/kg) of Δ^9-THC to produce tolerance to Δ^9-THC and WIN 55,212 hypothermic effects, which was comparable to that observed in the present study. These investigators also found that

TABLE 3

Effects of i.v. administered Δ^9-THC, CP 55,940 and WIN 55,212 on spontaneous activity, rectal temperature, antinociception and catalepsy in mice chronically treated with CP 55,940 (twice daily s.c. injections of 2 mg/kg for 13 days)

The ED_{50}'s were determined from dose-response curves constructed with a minimum of three doses and with at least 12 mice per dose.

			ED_{50} (mg/kg)			
Chronic Treatment	Days	Test	Hypoactivity	Hypothermia	Antinociception	Catalepsy
Vehicle	6	CP 55,940	0.018	0.081	0.033	0.31
CP 55,940	6	CP 55,940	3.065	8.05	***	5.90
Potency ratio			170*	99*		13.2*
Vehicle	13	CP 55,940	0.025	0.230	0.033	0.370
CP 55,940	13	CP 55,940	2.56	23.0	***	16.1
Potency ratio			102*	100*		44*
Vehicle	13	Δ^9-THC	2.90	3.31	1.58	3.85
CP 55,940	13	Δ^9-THC	17.7	15.9	24.5	25.1
Potency ratio			6.0**	4.8*	15.5*	6.5*
Vehicle	13	WIN 55,212	0.556	0.928	0.234	2.45
CP 55,940	13	WIN 55,212	6.29	76.4	4.39	36.8
Potency ratio			11.3**	82.3*	18.8*	15*

* ED_{50} was significantly different from the vehicle group at $P < .05$, and the shift is parallel.
** ED_{50} was significantly different from the vehicle group at $P < .05$, but the shift was not parallel.
*** No ED_{50} was obtained.

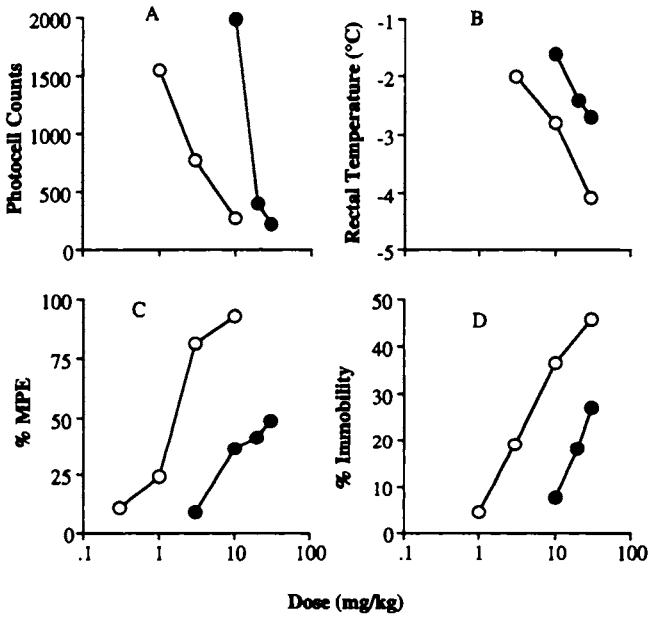

Fig. 6. Dose-response curves of i.v. administered Δ^9-THC on spontaneous activity (A), rectal temperature (B), antinociception (C) and catalepsy (D) in mice treated s.c. for 13 days with either vehicle (○) or 2 mg/kg CP 55,940 (●).

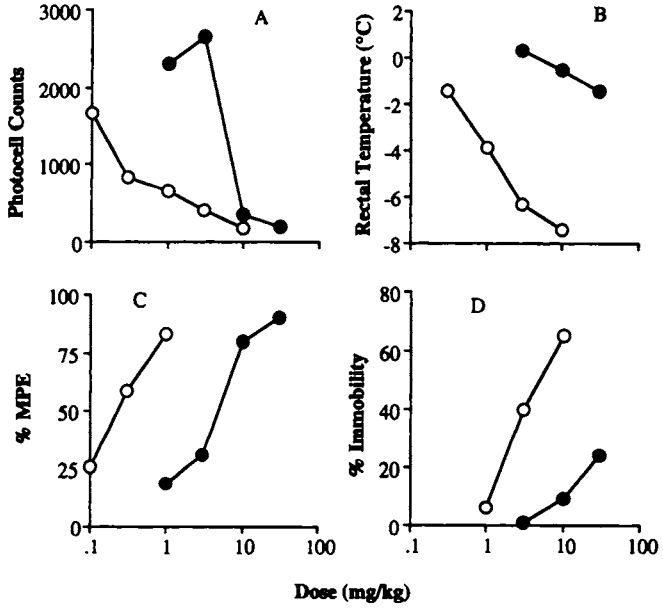

Fig. 7. Dose-response curves of i.v. administered WIN 55,212 on spontaneous activity (A), rectal temperature (B), antinociception (C) and catalepsy (D) in mice treated s.c. for 13 days with either vehicle (○) or CP 55,940 (●).

tolerance also developed to the hypothermic effects of CP 55,940, which was greater than that observed herein. Either protocol or mouse strain could account for this difference between these two studies.

The attenuation in the effects of Δ^9-THC and WIN 55,212 in CP 55,940-tolerant mice are consistent with the cross-tolerance found in Δ^9-THC tolerant mice. Significant tolerance was obtained to all four behavioral effects of Δ^9-THC in CP 55,940-tolerant mice. The effects of WIN 55,212 on spontaneous activity, hypothermia, antinociception and catalepsy also decreased in CP 55,940-tolerant mice to a considerable extent (11–82 times). These findings indicate that both CP 55,940 and Δ^9-THC can produce cross-tolerance to WIN 55,212. Due to insufficient quantities of WIN 55,212, it was not possible to conduct an investigation of the behavioral activity of Δ^9-THC and CP 55,940 in mice treated chronically with WIN 55,212. Although it is clear that tolerance develops to CP 55,940, Δ^9-THC and WIN 55,212 in CP 55,940-treated mice, some quantitative differences exist. With the exception of the hypothermic effect of WIN 55,212, the magnitude of tolerance to Δ^9-THC and WIN 55,212 is moderate (4.8–15 times), compared with those to CP 55,940 itself (44–102 times, excluding the anomalous tail-flick results).

Although the above studies demonstrate bidirectional cross-tolerance between Δ^9-THC and CP 55,940, it is also evident that the consequences of their repetitive administration is markedly different between them. The magnitude of the tolerance which develops to Δ^9-THC is comparable in both Δ^9-THC- and CP 55,940-treated mice, whereas it is not with CP 55,940. The differences in the magnitude of CP 55,940 tolerance in the two treatment groups is best illustrated by the rectal temperature and spontaneous activity

measures in which CP 55,940 tolerance was approximately 100 fold in the CP 55,940-treated group and less than 4 fold in the Δ^9-THC-treated groups. It has been proposed that the magnitude of tolerance that develops to the chronic administration of morphine is inversely related to spare receptor reserve (Porreca and Burks, 1983; Chavkin and Goldstein, 1984). Several studies have confirmed that several opioid agonists which are more potent than morphine produce a lesser degree of tolerance (Rosenbaum *et al.*, 1984; Miller *et al.*, 1986; Christie *et al.*, 1987; Stevens and Yaksh, 1989). Stevens and Yaksh (1989) demonstrated that chronic administration with equi-analgesic doses of opioids which varied in potency resulted in tolerance, the magnitude of which was inversely related to potency. These authors invoked the argument that more potent agonists activate fewer receptors and therefore produce less tolerance, a notion that is consistent with spare receptor theory. However, just the opposite occurred in the present study in that the more potent CP 55,940 produced the greatest amount of tolerance. Although efficacy of CP 55,940 has not been generally assessed *in vivo*, it is more efficacious than Δ^9-THC in inhibition of adenylyl cyclase (Howlett *et al.*, 1986). Recent studies (Oviedo *et al.*, 1993) conducted in rats revealed that 14-day administration with Δ^9-THC (10 mg/kg) and CP 55,940 (3 mg/kg) reduced the receptor B_{max} by 50% to 60% and produced tolerance that appeared to be comparable (tolerance was not quantified). Although it is difficult to compare the two studies because of differences in treatment protocols, species and behavioral measures, it would appear that tolerance to the cannabinoids develops differently in mice and rats. It would seem that if receptor down-regulation was responsible for tolerance development, then the magnitude of tolerance would be the same for both Δ^9-THC and CP 55,940. On the other hand, it is conceivable that CP 55,940 is interacting with a greater number of receptors than Δ^9-THC because CP 55,940 has a greater affinity for the receptor. In contrast to the opioid studies in which equi-active doses of the different agonists were evaluated in order to assess tolerance development, relatively inactive doses of CP 55,940 and Δ^9-THC were used in the present studies in order to avoid the complications of weight loss. However, it is important to point out that although the doses used for producing CP 55,940 and Δ^9-THC tolerance are inactive when given i.p., they were not in a ratio comparable to their i.v. potencies. CP 55,940 is at least 20 times more potent than Δ^9-THC; yet, the doses used differed by only five fold. It remains to be established whether a more rigorous treatment regimen can be developed for induction of Δ^9-THC tolerance comparable in magnitude to that produced by CP 55,940. It will be important to determine whether the development of tolerance to other cannabinoids is also directly related to potency. It will also be important to measure the spare receptor reserve for these cannabinoids.

It is possible that the explanation for the difference observed between Δ^9-THC and CP 55,940 is not due to differences in receptor activity, but rather to differences in intrinsic activity at the receptor resulting in differing efficacies *in vivo*. One possible explanation for the limited tolerance development following Δ^9-THC treatment is that it is a partial agonist. CP 55,940 would then apparently be a full agonist, which would explain the dose-response curves shifts in CP 55,940-tolerant mice. If true, the similarity between Δ^9-THC and WIN 55,212 in CP 55,940-tolerant animal would suggest that WIN 55,212 is also a partial agonist. The concept that Δ^9-THC is a partial agonist has been suggested elsewhere, but there are little data to support this contention. Behavioral evidence supporting this hypothesis include data indicating that the maximal effects of CP 55,940 for lowering rectal temperature and inducing catalepsy are greater than those of either Δ^9-THC or WIN 55,212 (Compton *et al.*, 1992b).

The mechanism of cannabinoid tolerance remains unclear. At least tolerance development to Δ^9-THC cannot be explained by altered pharmacokinetics (Martin *et al.*, 1976) and there is a lack of consensus regarding pharmacodynamic events. It has been shown that chronic exposure to Δ^9-THC fails to produce an irreversible alteration in brain cannabinoid receptors (Westlake *et al.*, 1991). Abood *et al.* (1993) also found no changes in cannabinoid binding between tolerant and nontolerant animals when whole brain homogenates were examined. In contrast, Oviedo *et al.* (1993) reported extensive receptor down-regulation in CP 55,940-tolerant rats. Dill and Howlett (1988) demonstrated that short-term exposure of N18TG2 neuroblastoma cells to Δ^9-THC produced an attenuation in the inhibitory effects of Δ^9-THC on adenylyl cyclase activity. It remains to be established whether receptor down-regulation or alterations in second messenger systems are responsible for the development of cannabinoid tolerance, or whether multiple systems (neuronal or second messenger) mediate different pharmacological events for which there are individual regulatory controls.

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