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Time course for the induction and maintenance of tolerance to Δ^9-tetrahydrocannabinol in mice

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Abstract

The time course for the development of tolerance to delta-9-tetrahydrocannabinol (Δ^9-THC) was studied in an effort to determine the role that length of dosing may have in the onset and maintenance of tolerance. Mice were chronically treated with either vehicle or 10 mg/kg of Δ^9-THC subcutaneously twice a day. The mice were tested 24 h after the last injection for tolerance as assessed by the production of antinociception and suppression of spontaneous activity. Tolerance was first observed after three injections of Δ^9-THC (1.5 days) resulting in a 7-fold and 23-fold decrease in potency for the measures of antinociception and hypoactivity, respectively. Seven injections of Δ^9-THC (3.5 days of dosing) resulted in a 12-fold and 36-fold decrease in potency, respectively, while 13 injections of Δ^9-THC (6.5 days of dosing) produced a 6.2-fold and 9.8-fold degree of tolerance. The time course for the recovery from Δ^9-THC-induced tolerance was also determined with a separate group of animals. Mice were dosed for 6.5 days with 10 mg/kg of Δ^9-THC and were not tested until 4.5, 7.5, and 11.5 days after cessation of drug treatment. After 4.5 days without drug treatment the mice exhibited a 7.5-fold and 2.3-fold degree of tolerance as measured by antinociception and hypoactivity, respectively. After 7.5 days without drug treatment a 3.4-fold degree of tolerance remained for the measure of antinociception, while no tolerance was detected for the measure of hypoactivity. No tolerance was observed for the measure of antinociception after 11.5 days without drug treatment. This time course indicates that the mechanisms responsible for either the production or maintenance of tolerance differ between the measures of antinociception and suppression of spontaneous activity.

Keywords: Delta-9-tetrahydrocannabinol; Δ^9-THC ; Cannabinoid; Tolerance; Time course

1. Introduction

Delta-9-tetrahydrocannabinol (Δ^9-THC) is the major psychoactive component of marijuana (Mechoulam et al., 1970). The effects of this compound have been under scrutiny for years, however, the molecular mechanisms underlying these events have only recently been identified. This is primarily due to the discovery of two cannabinoid receptors designated CB_1 (Matsuda et al., 1990) and CB_2 (Munro et al., 1993). The discovery of these receptors as well as two putative endogenous ligands, anandamide (Devane et al., 1992) and 2-arachidonyl glycerol (Mechoulam et al., 1995), and the development of a CB_1 receptor antagonist (Rinaldi-Carmona et al., 1994) have greatly advanced the understanding of how these receptors produce the numerous effects of Δ^9-THC. Some of the basic roles of the CB_1 receptor and it's subsequent signal transduction cascades have been elucidated, including the linkage of this receptor to inhibition of adenylyl cyclase (Howlett, 1984) and to the MAP kinase system (Bouaboula et al., 1995). CB_1 receptors play a role in pain modulation as evidenced by their ability to initiate the release of endogenous opioids (Pugh et al., 1996).

Recent endeavors have focused on understanding the regulation of the CB_1 receptor in an effort to more clearly define the role of the cannabinoid system in the

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central nervous system. The ability of Δ^9-THC to readily produce tolerance that is easily measured (McMillan et al., 1971) has led to the use of this phenomena as a tool to study the biology of the cannabinoid receptor. This method is ideal for studying the regulation of the cannabinoid receptor as it initiates natural regulatory processes of endogenous cannabinoid systems in an intact animal. It has been known for some time that tolerance to Δ^9-THC occurs in numerous species, including rodents (Carlini, 1968), monkeys (Sassenrath and Chapman, 1976), pigeons (McMillan et al., 1970), and dogs (Kaymakcalan et al., 1974). These studies demonstrated tolerance to Δ^9-THC through the use of a variety of protocols. However, the data generated from many Δ^9-THC tolerance studies have often been conflicting and ambiguous. This may be due in part to the manner in which tolerance was produced. The protocols often varied in the dose of Δ^9-THC, the route of administration, and the number and timing of administrations. One important facet of the different dosing protocols, and a possible source for descrepancies among studies, involves the length of exposure to Δ^9-THC during tolerance production.

In order to ascertain the effect that length of exposure to Δ^9-THC may have on the production and degree of tolerance generated, we sought to determine the time course through which Δ^9-THC induces tolerance. This effort will not only allow the quantification of the degree of tolerance to Δ^9-THC but will also help determine when the onset of tolerance occurs as well as when full tolerance is reached. The time required for Δ^9-THC-induced tolerance to abate was also determined. The mouse was chosen as an appropriate model for these experiments due to their ease of maintenance and the ability of Δ^9-THC to produce a distinctive profile of effects. This profile includes antinociception, hypomotility, catalepsy, and hypothermia (Martin et al., 1991). Initial studies in this laboratory have determined that Δ^9-THC administered twice a day for 6.5 days produces a robust and reliable tolerance to these four measures in mice (Abood et al., 1993; Fan et al., 1994). This established protocol was modified to determine the time course of Δ^9-THC-induced tolerance for this study.

2. Materials and methods

2.1. Subjects

Male ICR mice (Harlan Laboratories, Indianapolis, IN) weighing between 24 and 30 g were used in all experiments. Mice were maintained on a 14:10 h light/dark cycle with food and water available ad lib. Δ^9-THC was obtained from the National Institute on Drug Abuse (Bethesda, MD).

2.2. Drug preparation and chronic administration

Δ^9-THC was dissolved in a 1:1:18 solution of ethanol, emulphor and saline, respectively. The mice were injected subcutaneously once in the morning and once in the afternoon (approximately 09:00 and 16:00 h) for varying lengths of time to establish the time course of Δ^9-THC-induced tolerance. On the last day of dosing, the mice received a morning injection only. Twenty-four hours later the mice were challenged with a single i.v. dose of either Δ^9-THC or vehicle in the tail vein followed by behavioral assays to assess tolerance. Dose–response curves were generated and the resultant ED_{50} values and potency ratios were calculated. Animals were treated for either 1, 3, or 6 days of full morning and afternoon dosing followed by one morning injection. These regimens are represented as days 1.5, 3.5, and 6.5, respectively. Day 0.5 represents one dose in the morning only followed by testing 24 h later.

The time course by which Δ^9-THC-induced tolerance subsides was assessed by dosing for 6.5 days followed by 4.5, 7.5, or 11.5 days without drug treatment. The mice were challenged after this recovery period with an i.v. injection of Δ^9-THC in the tail vein. Again, dose–response curves, ED_{50} values, and potency ratios were generated.

2.3. Behavioral evaluations

All animals were allowed to acclimate to the observation room overnight. Twenty-four hours after the last injection the mice in both drug- and vehicle-treated groups received a challenge dose of Δ^9-THC and were evaluated for hypomotility and antinociception. The 24-h time period was chosen in an effort to allow the animal to clear any residual drug from the last injection. Antinociception was determined using the tail-flick reaction time to a heat stimulus (Dewey et al., 1970). The baseline latency period (2–4 s) was first determined prior to i.v. administration of vehicle or drug. The mice were then administered Δ^9-THC through tail vein injection and placed in individual photocell activity chambers 5 min later. Spontaneous activity was monitored for 10 min in a Digiscan Animal Activity Monitor (Omnitech Electronic Inc., Columbus, OH) as measured by the number of interruptions of 16 photocell beams per chamber. The mice were then assessed at 20 min post-injection for tail-flick latency and the difference in control and test latencies were calculated. A 10-s maximum latency was used in order to avoid tail injury.

2.4. Data analysis

Suppression of spontaneous activity was expressed as the percentage of the activity of mice treated repeti-

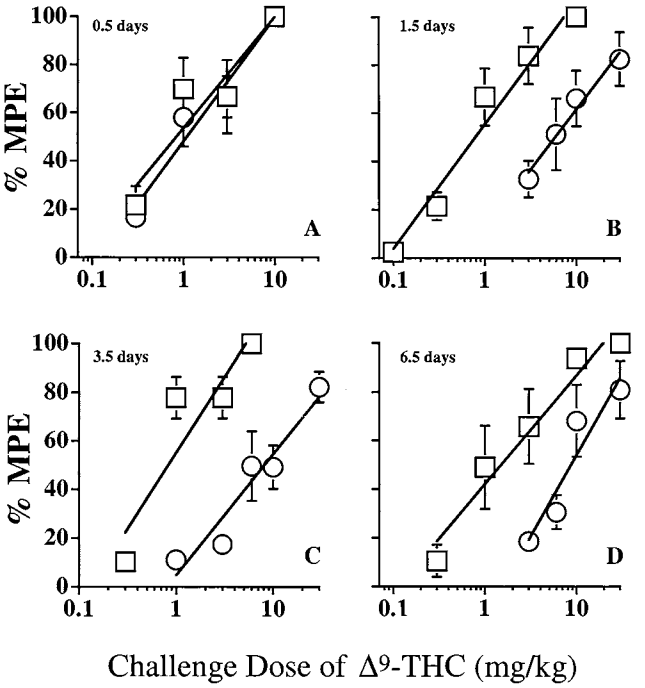

Fig. 1. Time course for the development of tolerance to the antinociceptive effects of Δ^9-THC. Each point represents the mean percent MPE ($\pm$S.E.) of 6–12 mice treated chronically with vehicle (□) or Δ^9-THC (○) for 0.5 days (A), 1.5 days (B), 3.5 days (C) or 6.5 days (D).

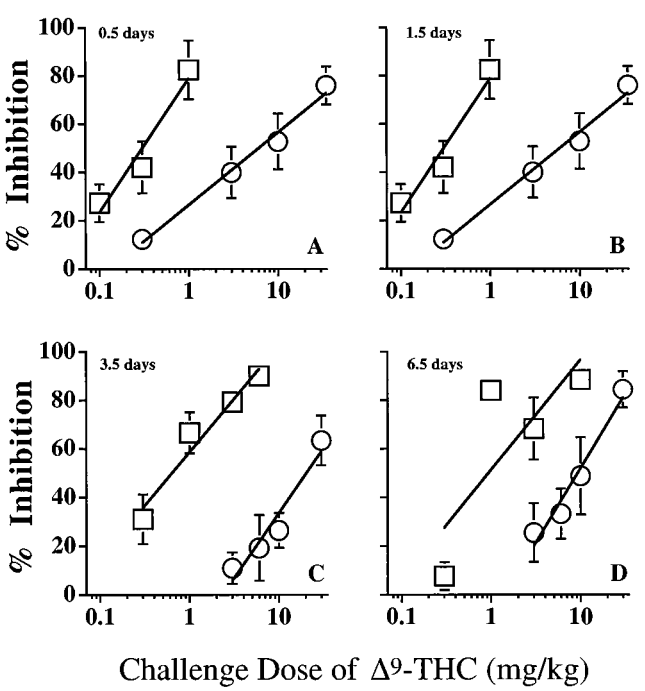

Fig. 2. Time course for the development of tolerance to the Δ^9-THC-induced hypoactivity. Each point represents the mean percent inhibition ($\pm$S.E.) of 6–12 mice treated chronically with vehicle (□) or Δ^9-THC (○) for 0.5 days (A), 1.5 days (B), 3.5 days (C) or 6.5 days (D).

tively with vehicle and challenged with vehicle. Antinociception was expressed as percent of maximum possible effect (% MPE = [(test latency − control latency)/(10 s − control latency)] × 100). Dose–response curves were generated based upon increasing Δ^9-THC challenge doses in groups of 6–12 mice. ED_{50} values were calculated based upon least squares linear regression followed by calculation of 95% confidence limits (Bliss, 1967). A shift in dose–response curves is considered significant when there is no overlap of the ED_{50} 95% confidence limits between mice chronically treated with vehicle and those chronically treated with Δ^9-THC. The potency ratio was determined based on the methods of Colquhoun (1971). A potency ratio is considered significant when the lower 95% confidence limit is >1.

3. Results

3.1. Induction of tolerance to Δ^9-THC

Figs. 1 and 2 present the dose–response curves for the time course of the onset of Δ^9-THC-induced tolerance for the measures of antinociception and hypoactivity, respectively. Dosing for the 6.5-day time period produced a large degree of tolerance in both measures (panels D, Figs. 1 and 2). Analysis of dose–response curves generated from these animals determined that those chronically treated with Δ^9-THC for 6.5 days had a significant shift in ED_{50} value and a significant potency ratio (Table 1), confirming that the animals were indeed tolerant. Subsequent experiments, with results depicted in Figs. 1 and 2 and summarized in Table 1, demonstrate that tolerance was produced with 3.5 and 1.5 days of dosing as well. However, pretreatment of a single dose of Δ^9-THC (0.5 days) did not produce any significant difference in potency ratio or ED_{50} values. The time course for the production of tolerance to the antinociceptive effects of Δ^9-THC was comparable to the time course for the development of tolerance to the hypoactivity effects of Δ^9-THC. The baseline ED_{50} for animals chronically treated with vehicle remained stable for each time point across both measures. For the measure of antinociception, the ED_{50} increased from 1.1 mg/kg Δ^9-THC for day 0.5 to 5.75 and 8.76 mg/kg for days 1.5 and 3.5, respectively. However, the ED_{50} did not increase between day 3.5 and 6.5 for the measure of antinociception. The same trend was observed with increasing dosing length for the measure of hypoactivity; however, the 6.5-day time point actually produced less of a shift in ED_{50} value than the 3.5-day time point. As expected, potency ratios confirmed the changes in the ED_{50} values. An increase in the potency ratio of Δ^9-THC was observed with increasing dosing length for both measures on days 0.5, 1.5, and 3.5. The

Table 1
Effects of increasing length of drug dosing on the production of tolerance to the Δ^9-THC-induced antinociception and hypoactivity

Day	Antinociception			Hypoactivity		
	ED_{50} (mg/kg)		Potency ratio	ED_{50} (mg/kg)		Potency ratio
	Vehicle	Δ^9-THC		Vehicle	Δ^9-THC	
0.5	0.81 (0.42–1.55)	1.10 (0.75–1.63)	1.49 (0.64–3.42)	0.46 (0.16–1.35)	0.61 (0.25–1.49)	1.33 (0.60–3.08)
1.5	0.78 (0.50–1.20)	5.75 (3.20–10.3)[a]	7.48 (3.62–15.4)[b]	0.26 (0.18–0.37)	4.16 (1.94–8.93)[a]	23.4 (8.16–69.6)[b]
3.5	0.73 (0.48–1.12)	8.76 (6.18–12.4)[a]	12.4 (8.21–21.3)[b]	0.47 (0.21–1.05)	17.0 (11.4–25.3)[a]	36.1 (24.4–87.3)[b]
6.5	1.50 (0.86–2.60)	9.14 (6.55–12.7)[a]	6.22 (3.40–13.3)[b]	0.80 (0.40–1.61)	8.09 (5.01–13.1)[a]	9.79 (5.13–25.5)[b]

[a] Denotes a significant shift in the dose–response curve as indicated by confidence limits that do not overlap between the vehicle and Δ^9-THC-treated groups.
[b] Denotes a significant potency ratio as defined by a lower 95% confidence limit above 1.

potency ratio for day 6.5 was less than that of day 3.5 for the measure of antinociception and less than 1.5 and 3.5 for hypoactivity.

3.2. Reversal from Δ^9-THC-induced tolerance

Recovery studies were performed by dosing the mice for 6.5 days followed by cessation of dosing for varying periods of time before testing. The mice were then challenged with Δ^9-THC and dose–response curves and potency ratios were generated. Results are depicted in Figs. 3 and 4 and summarized in Table 2. After 6.5 days of dosing, the potency ratio for Δ^9-THC was 6.22 for the measure of antinociception (Table 1). Four and one-half days after cessation of drug dosing, the potency ratio increased to 7.57. The potency ratio decreased from 9.79 to 2.34 four and one-half days after cessation of drug treatment for the measure of hypoactivity (Table 1). However, while the degree of tolerance assessed at this time point did decrease from the 6.5 day time point for both measures, the shift in ED_{50} values between those animals receiving repetitive vehicle and those dosed with Δ^9-THC was still significant. After 7.5 days of recovery the potency ratio dropped even further to 3.35 for the measure of antinociception, yet the shift in ED_{50} values between the drug and vehicle treated groups was significant. However, after 7.5 days without drug dosing, tolerance to the Δ^9-THC-induced hypoactivity completely disappeared with a drop in potency ratio to 1.08 with a lower 95% confidence limit of 0.96. These findings indicate that the animals were still tolerant to the antinociceptive effects of Δ^9-THC but not to its ability to suppress spontaneous activity. Full recovery did not occur for the measure of antinociception until 11.5 days after cessation of drug treatment in which the potency ratio dropped to 0.95 with a lower 95% confidence limit of 0.51, thereby demonstrating that the animals were not tolerant.

4. Discussion

It is commonly believed that the array of effects produced by cannabinoids results from the activation of distinct neuronal pathways or systems. If the effects of Δ^9-THC are the result of CB_1 receptor occupancy, then it is important to determine what differences exist between such systems. Therefore, while most research focuses on receptor occupancy and changes occurring in tolerant animals, we have chosen to examine the onset and offset of Δ^9-THC-induced tolerance in an effort to more fully understand the way in which tolerance develops.

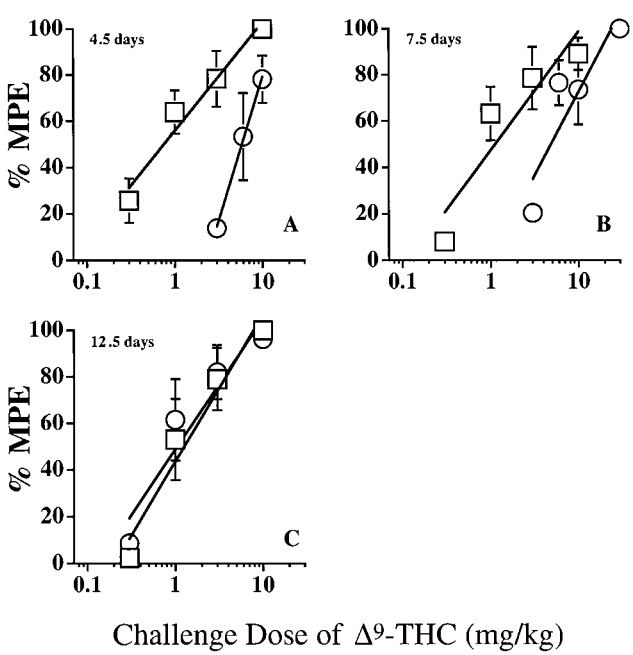

Fig. 3. Time course for tolerance to Δ^9-THC-induced antinociception to disappear. Each point represents the mean percent MPE ($\pm$S.E.) of 6–12 mice treated chronically with vehicle (□) or Δ^9-THC (○) for 6.5 days followed by cessation of treatment for 4.5 (A), 7.5 (B), and 12.5 (C) days before testing.

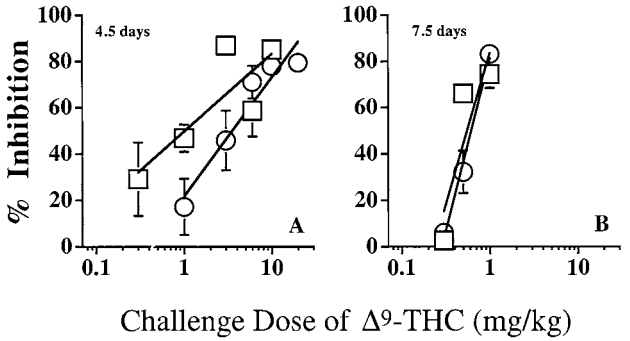

Fig. 4. Time course for tolerance to Δ^9-THC-induced hypoactivity to disappear. Each point represents the mean percent inhibition (± S.E.) of 6–12 mice treated chronically with vehicle (□) or Δ^9-THC (○) for 6.5 days and allowed to recover for 4.5 days (A) or 7.5 days (B).

Our study indicates that a difference does exist in the production and maintenance of tolerance between cannabinoid-induced antinociception and hypoactivity. Such differences in the time-course of cannabinoid action may account for a number of discrepancies in the current state of cannabinoid receptor biology. Despite the large quantity of data generated through the use of cannabinoid-induced tolerance to regulate CB_1 function, a clear trend that describes the association of tolerance to CB_1 binding sites and mRNA levels has yet to be determined. The results from many different laboratories have in fact often conflicted with one another. For example, Zhuang et al. (1998) observed an increase in CB_1 receptor mRNA in the cerebellum while another study failed to observe any changes in CB_1 mRNA in the cerebellum (Romero et al., 1997). It has also been reported that rats tolerant to Δ^9-THC exhibit a decrease in the B_{max} of the CB_1 receptor in the striatum (Rodriguez de Fonseca et al., 1994), while other laboratories do not detect any change in the B_{max} (Westlake et al., 1991). One possible explanation for this lack of consensus may be a difference in the degree of tolerance produced between studies. The difference in the length of dosing between studies may produce different levels of tolerance. It is likely that such variations would result in the production of a different profile of molecular events. There are few data describing the quantity and quality of tolerance produced through the many protocols in use to induce tolerance to Δ^9-THC. We have attempted to describe in quantitative terms the time course by which Δ^9-THC induces tolerance in an effort to ascertain the onset of tolerance and the nature of the resultant tolerance over time. The data indicate that tolerance to Δ^9-THC occurs in a systematic fashion, with the onset of tolerance occurring within 1.5 days of dosing. The level of tolerance increases with 3.5 days of dosing for both measures as indicated by changes in potency ratios. Six and a half days of dosing results in no further elevation of tolerance from the 3.5-day time point for the measure of antinociception and a decrease below this point for hypoactivity. These results are consistent with previous studies performed in this laboratory (Fan et al., 1994). The study found that the 6.5-day regimen resulted in an ED_{50} of 10.7 and 23.8 mg/kg for the measures of antinociception and hypoactivity, respectively. These values compare relatively well with the ED_{50} values of 9.14 and 8.09 mg/kg reported herein. The discrepancy between the ED_{50} values for the measure of hypoactivity may indicate a higher level of variability in this particular measure. This discrepancy is difficult to evaluate, as the Fan study did not include 95% confidence limits. It is important to note that the ED_{50} levels of the vehicle control group remained stable between all time point and measures in our study, indicating that variation between experiments was rather minor. The Fan et al. (1994) study further extended the length of dosing to 13.5 days, resulting in 9.5 and 13.3 mg/kg ED_{50} values for the measures of antinociception and hypoactivity,

Table 2
Recovery from Δ^9-THC-induced tolerance

Days of recovery	Antinociception			Hypoactivity		
	ED_{50}		Potency ratio	ED_{50}		Potency ratio
	Vehicle	Δ^9-THC		Vehicle	Δ^9-THC	
4.5	0.68 (0.36–1.30)	5.81 (4.33–7.80)[a]	7.57 (3.64–20.7)[b]	0.80 (0.45–1.41)	2.49 (1.74–3.55)[a]	2.34 (1.29–4.05)[b]
7.5	0.95 (0.51–1.79)	3.91 (2.51–6.10)[a]	3.35 (1.50–6.76)[b]	0.53 (0.47–0.60)	0.57 (0.51–0.63)	1.08 (0.96–1.22)
11.5	1.24 (0.78–1.98)	1.08 (0.68–1.72)	0.95 (0.51–1.70)	NA	NA	NA

[a] Denotes a significant shift in the dose–response curve as indicated by confidence limits that do not overlap between the vehicle and Δ^9-THC-treated groups.

[b] Denotes a significant potency ratio as defined by a lower 95% confidence limit above 1.

respectively. This is quite similar to the 9.14 and 8.09 mg/kg ED_{50} values resulting from 6.5 days of dosing in our study and further confirms our postulation that tolerance does not increase after the 3.5-day time point.

Our results are supported by a recent study performed by Zhuang et al. (1998) which found that changes in the CB_1 mRNA levels of three different brain regions occurred within one day of Δ^9-THC. Although a different dosing schedule was used, the approximate initiation of Δ^9-THC-induced molecular changes corresponds with the onset of tolerance in our studies. It has also been demonstrated in our laboratory that dosing for 6.5 days with CP-55 940, a potent cannabinoid analog, results in a decrease in CB_1 receptor binding sites in the cerebellum as well as an increase in CB_1 receptor mRNA (Fan et al., 1996). Presumably, the increase in receptor mRNA may be a compensatory mechanism to counteract the decrease in CB_1 binding sites. The decrease in the degree of tolerance observed in our study between the 3.5 and 6.5-day time points may be a result of such a compensatory mechanism. However, it should be noted that although the same dosing schedule was used, a difference may exist between the tolerance induced by Δ^9-THC and that induced by other cannabinoids, including CP-55 940. Nonetheless, it appears that length of dosing plays a role not only in the induction of tolerance but also in the degree of tolerance produced. In determining the molecular events involved in Δ^9-THC-induced tolerance, such dosing factors should be taken into account.

The offset of Δ^9-THC-induced tolerance occurred within 7.5 days of cessation of drug treatment for the measure of hypoactivity and 11.5 days for the measure of antinociception. This divergence in the time course of recovery between the two measures raises a number of possibilities for discerning the molecular events involved in the development and maintenance of tolerance. It is quite possible that the mechanisms responsible for the development or maintenance of tolerance to the antinociceptive effects of Δ^9-THC are different from those responsible for its hypoactive effects. Any future studies into the molecular events responsible for Δ^9-THC-induced tolerance will have to consider the possibility that molecular changes involved with the onset of tolerance may differ greatly from those responsible for maintenance or even recovery from tolerance.

Acknowledgements

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