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Rapid tolerance to Δ^9-tetrahydrocannabinol and cross-tolerance between ethanol and Δ^9-tetrahydrocannabinol in mice

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Abstract

Motor incoordination in the rota-rod test was used to assess the development of rapid tolerance to Δ^9-tetrahydrocannabinol and rapid cross-tolerance between ethanol and Δ^9-tetrahydrocannabinol in mice. Further, the influence of the cannabinoid receptor antagonist SR 141716A (*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxyamide) on the motor impairment induced by both drugs was examined. Mice were injected on day 1 with equipotent doses of Δ^9-tetrahydrocannabinol (28 mg/kg, i.p.) and ethanol (2.25 g/kg, i.p.) and tested at 30, 60 and 90 min after the injections. On day 2, control groups received ethanol or Δ^9-tetrahydrocannabinol, some groups received the same treatment as the day before, while the remaining groups switched the treatment. All groups were tested to evaluate tolerance. The development of rapid tolerance to Δ^9-tetrahydrocannabinol was observed and pretreatment with ethanol resulted in rapid cross-tolerance to Δ^9-tetrahydrocannabinol. SR 141716A (2 mg/kg, i.p.) failed to block the development of rapid tolerance to both drugs, ethanol and Δ^9-tetrahydrocannabinol. These results suggest that Δ^9-tetrahydrocannabinol, similarly to ethanol, can induce rapid tolerance to motor incoordination in mice. They also support the use of the 2-day protocol as an effective procedure to reduce the length of drug exposure necessary to induce tolerance.

Keywords: Rapid tolerance; Cross-tolerance; Rota-rod; Δ^9-Tetrahydrocannabinol; Ethanol; SR 141716A

1. Introduction

The repeated exposure to *Cannabis sativa* or its principal psychoactive constituent, Δ^9-tetrahydrocannabinol, has been reported to induce tolerance in several species, including mice, using different measures such as hypothermia, antinociception, catalepsy, depression of locomotor activity, hypotension, anticonvulsant activity, corticosteroid release and schedule-controlled behavior (reviewed by Adams and Martin, 1996). However, within the last decade, the research on cannabis has undergone dramatic advances due to developments such as the identification, cloning and expression of selective cannabinoid CB_1 and CB_2 receptors (Matsuda et al., 1990; Munro et al., 1993), the isolation of the endogenous cannabinoid receptor ligands anandamide and 2-arachidonyl glycerol (Devane et al., 1992; Stella et al., 1997) and, most recently, the availability of a selective cannabinoid CB_1 receptor antagonist, SR 141716A (Rinaldi-Carmona et al., 1994).

Although drug tolerance, in many situations, is rather easily demonstrated and measured, there is an increasing awareness of the fact that the development of this response is a complex event which is influenced by both behavioral and environmental factors. For instance, there are several reports showing that tolerance to Δ^9-tetrahydrocannabinol occurs in different species, but many of these studies have been conflicting and ambiguous (Carlini, 1968; McMillan et al., 1970; Kaymakcalan et al., 1974). As mentioned recently by Bass and Martin (2000), a possible source for discrepancies among studies may be the length of exposure to Δ^9-tetrahydrocannabinol during tolerance induction. Moreover, studies designed to assess the rate of development of chronic tolerance to ethanol indicate that this rate is highly dependent upon the test measure examined (Bitrán and Kalant, 1991). The phenomenon designated as rapid tolerance to ethanol has been reported to occur within 8–24 h, after the effect of the first dose has disappeared (Crabbe et al., 1979). One of the most interesting aspects of rapid tolerance is that the magnitude of the observed tolerance is often not significantly different from that observed following chronic ethanol treatment (Khanna et al., 1997). In addition, both rapid tolerance and chronic

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tolerance have been shown to be influenced by, or to require, the activation of a process related to learning and memory (Bitrán and Kalant, 1991).

Alcohol and marijuana are two of the most widely used psychoactive drugs in the world. There is a growing literature, mainly consisting of human studies, documenting that Δ^9-tetrahydrocannabinol and ethanol share some central nervous system (CNS) depressant effects (Hollister and Gillespie, 1970; Ng et al., 1973) and that there are certain doses of these drugs that may produce comparable behavioral effects (Heishman et al., 1997). It is important to note in the context of the present study the early findings of Sprague and Craigmill (1976) that ethanol and Δ^9-tetrahydrocannabinol injected over 12 and 7 days, respectively, produce a similar type of behavioral impairment in mouse performance measured on a rota-rod apparatus as well as the development of cross-tolerance between these two drugs.

The cannabinoid CB_1 receptor antagonist SR 141716A has been reported to antagonize many of the acute effects of cannabinoids including hypothermia, analgesia and locomotor activity (Rinaldi-Carmona et al., 1994; Compton et al., 1996). Following chronic Δ^9-tetrahydrocannabinol administration, SR 141716A precipitated a withdrawal syndrome in rats, mice and dogs (Cook et al., 1998; Lichtman et al., 1998). In addition, there are studies presumably investigating the role of the cannabinoid CB_1 receptor in the reinforcing effects of ethanol (Arnone et al., 1997; Colombo et al., 1998).

In view of the above, the purpose of the present study was to examine whether, similarly to ethanol, a rapid model for the development of tolerance to Δ^9-tetrahydrocannabinol-induced motor impairment in mice could be achieved. To explore this possibility, we have compared a motor-impairing doses of Δ^9-tetrahydrocannabinol and ethanol on the rota-rod test. In addition, the development of rapid cross-tolerance between these two drugs was assessed using the same test. Further, the influence of a cannabinoid CB_1 receptor antagonist on the development of rapid tolerance by ethanol and Δ^9-tetrahydrocannabinol was investigated.

2. Materials and methods

2.1. Animals

Male Swiss albino mice (25–35 g, 6–8 weeks old) were maintained in groups in the colony room of the Department of Pharmacology, under a light–dark cycle of 12 h (lights on at 6:00 a.m.) with food and water ad libitum. All procedures used in the present study complied with the guidelines on animal care of the Brazilian Society of Neuroscience and Behavior.

2.2. Drugs

Ethanol was purchased from Merck (Darmstadt, Germany). Δ^9-Tetrahydrocannabinol was obtained from the National Institute of Drug Abuse (Bethesda, USA) at 200 mg/ml in alcohol. SR 141716A (*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxyamide) was kindly supplied by Sanofi Recherche (Montpellier, France). Ethanol was diluted with saline to a concentration of 14% w/v. The appropriate concentration of Δ^9-tetrahydrocannabinol was prepared immediately before use by evaporating the alcohol and emulsifying the residue with Tween-80 (Takahashi and Singer, 1979). One drop of Tween-80 per 10 ml was added for the preparation of the SR 141716A suspension. Control solution was prepared with the corresponding vehicle. All solutions were administered by i.p. route.

2.3. Procedure

Motor impairment was measured on a rota-rod apparatus (Rotamex-V-EE/85; Columbus Instruments, USA) controlled through a computer and as described previously (Barreto et al., 1998). Briefly, mice were trained under continuous acceleration (1 rpm/s) in 1-min sessions. Whenever the animal falls off the rotating bar, it receives a foot shock (0.3 mA). The speed of the rotating bar when the animal falls off is taken as the performance score. Animals that did not reach a stable baseline (at least 20 s) in 15 trials were discarded. After the selection, experimental and control groups ($N = 10$) were matched according to their body weight and mean performance during the last session. With this procedure, animals presented similar baseline values on both days for all groups.

2.3.1. Effect of different doses of Δ^9-tetrahydrocannabinol on the development of rapid tolerance

On day 1, four groups of trained mice received different doses of Δ^9-tetrahydrocannabinol (8, 15 or 28 mg/kg) or control solution. Then, they were tested on the rota-rod 30, 60 and 90 min later. Mice were then returned to their home cages. On day 2, each animal received exactly the same treatment that it received on the previous day, and 30, 60 and 90 min later, all animals were tested on the rota-rod to evaluate the development of rapid tolerance.

2.3.2. Comparison between the development of rapid tolerance to ethanol and Δ^9-tetrahydrocannabinol and cross-tolerance between these drugs

Previous experiments in this laboratory have shown that a single dose of 2.25 g/kg ethanol caused a significant tolerance in this paradigm (Barbosa and Morato, 2001). Therefore, ethanol (2.25 g/kg) and Δ^9-tetrahydrocannabinol (28 mg/kg) were considered equipotent in terms of

rapid tolerance response on the rota-rod and both doses were selected for the cross-tolerance experiment.

On day 1, two groups of trained mice received control solution, two groups received ethanol (2.25 g/kg) and two groups received Δ^9-tetrahydrocannabinol (28 mg/kg). Then, they were tested on the rota-rod 30, 60 and 90 min later. Mice were then returned to their home cages. On day 2, the groups treated with control solution on day 1 received either ethanol or Δ^9-tetrahydrocannabinol. Groups that had received ethanol or Δ^9-tetrahydrocannabinol on day 1 (one group each) received the same treatment on day 2. The remaining two groups (one ethanol and one Δ^9-tetrahydrocannabinol group from day 1) switched their treatments on day 2. All groups were tested on the rota-rod to evaluate tolerance 30, 60 and 90 min later.

2.3.3. Influence of a cannabinoid CB_1 receptor antagonist on the development of rapid tolerance to ethanol and Δ^9-tetrahydrocannabinol

On day 1, eight groups of trained mice were assigned to subgroups pretreated with control solution (four subgroups) or SR 141716A (2 mg/kg; four subgroups). Ten minutes later, half of each subgroup received ethanol (2.25 g/kg) or Δ^9-tetrahydrocannabinol (28 mg/kg), and the remaining subgroups received control solution. Then, they were tested on the rota-rod 30, 60 and 90 min later. On day 2, a challenge dose of ethanol or Δ^9-tetrahydrocannabinol was given to assess rapid tolerance.

2.4. Statistical analysis

Data were analyzed using one-way or two-way analyses of variance (ANOVA) for repeated measures. Subsequent comparisons were done using Tukey's Least Significant Difference (LSD) test. Student's *t*-test was used to detect differences between two means in the Δ^9-tetrahydrocannabinol dose–response experiment group. Values of $P < 0.05$ were considered significant.

3. Results

3.1. Effect of different doses of Δ^9-tetrahydrocannabinol on the development of rapid tolerance

The results of this experiment are shown in Fig. 1. As can be seen, only the highest dose of Δ^9-tetrahydrocannabinol (28 mg/kg) induced a significant motor impairment compared to the control group, on day 1 ($P < 0.05$, Student's *t*-test). On day 2, animals that had received Δ^9-tetrahydrocannabinol (28 mg/kg) were tolerant to the motor impairment induced by the drug ($P < 0.05$, Student's *t*-test). This indicates rapid development of tolerance to Δ^9-tetrahydrocannabinol. The motor incoordination was most severe at 30 min following the injections. Thus, the highest dose of Δ^9-tetrahydrocannabinol (28 mg/kg) and the time point of 30 min were chosen to depict the subsequent results.

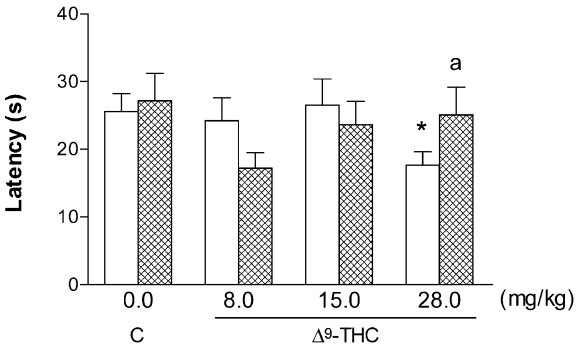

Fig. 1. Development of rapid tolerance to motor impairment induced by different doses of Δ^9-tetrahydrocannabinol (8, 15 or 28 mg/kg, i.p.) in mice, tested on the rota-rod apparatus. On day 1 (open bars), the control group (C) received control solution and the other groups received Δ^9-tetrahydrocannabinol. Animals were tested 30, 60 and 90 min after the injections. On day 2 (closed bars), the animals received the same treatment as in the previous day and were tested again. Results shown are means ± S.E.M. of 7–12 animals per group at the 30-min time point. $^a P < 0.05$, compared to respective day 1 result, and $^* P < 0.05$, compared to day 1 control group (Student's *t*-test).

3.2. Comparison between the development of rapid tolerance to ethanol and Δ^9-tetrahydrocannabinol and cross-tolerance between these drugs

The development of rapid tolerance to ethanol and rapid cross-tolerance to Δ^9-tetrahydrocannabinol is shown in Fig. 2A. A two-way analysis of variance indicated a significant main effect of treatment, $F(2, 22) = 8.56$, $P < 0.002$ and a significant interaction of day and treatment, $F(2, 22) = 52.05$, $P < 0.00001$. Post hoc comparisons revealed that on day 1, ethanol induced significant motor impairment in mice compared to the control group (LSD test, $P < 0.05$). On day 2, the same pattern of response was observed in the control group challenged with ethanol (control + ethanol group). However, animals which had received ethanol were tolerant to motor impairment induced by the drug (ethanol + ethanol group; LSD test, $P < 0.05$). The administration of Δ^9-tetrahydrocannabinol in mice previously treated with ethanol did not produce any motor impairment, relative to day 1 control baseline, thus indicating the development of rapid cross-tolerance from ethanol to Δ^9-tetrahydrocannabinol (Fig. 2A).

Fig. 2B depicts the results of the experiment on the development of rapid tolerance to Δ^9-tetrahydrocannabinol. A similar two-way analysis of variance indicated a significant effect of treatment, $F(2, 25) = 3.89$, $P < 0.03$, and the interaction of day and treatment, $F(2, 25) = 9.98$, $P < 0.0006$. Subsequent comparisons showed that on day 1, Δ^9-tetrahydrocannabinol induced a significant motor

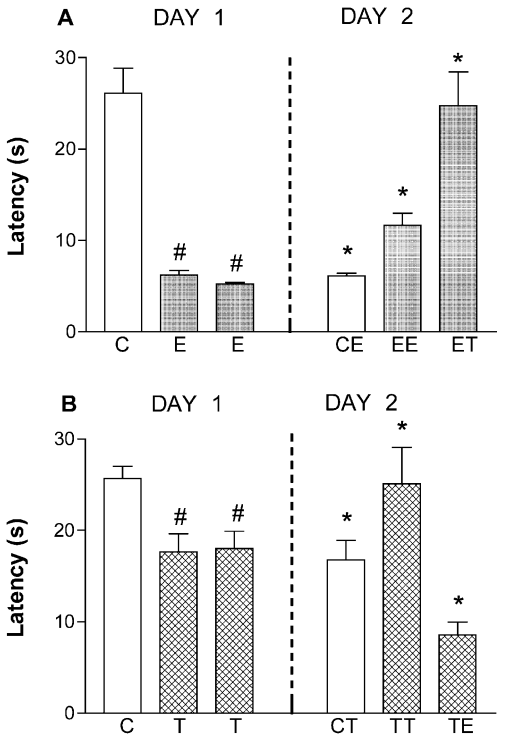

Fig. 2. Development of rapid tolerance and cross-tolerance to ethanol or Δ^9-tetrahydrocannabinol based on rota-rod performance. (A) On day 1, one group received control solution (C) and the other groups (E) received ethanol (2.25 g/kg). Animals were tested 30 min after treatment. On day 2, two groups (CE and EE) received a challenge dose of ethanol and the other group (ET) received Δ^9-tetrahydrocannabinol (28 mg/kg), then all animals were tested again. (B) On day 1, one group received control solution (C) and the other groups (T) received Δ^9-tetrahydrocannabinol (28 mg/kg). On day 2, two groups (CT and TT) received a challenge dose of Δ^9-tetrahydrocannabinol and the other group (TE) received ethanol (2.25 g/kg). Results shown are means ± S.E.M. of 8–10 animals per group. (#) Indicates statistically significant difference compared to control group; (*) indicates significant difference from corresponding groups on day 1 (LSD test).

impairment in mice tested on the rota-rod compared to the control group (LSD test, $P < 0.05$). Mice pretreated with Δ^9-tetrahydrocannabinol showed significantly less motor impairment in response to the challenge dose of the drug on day 2 (Δ^9-tetrahydrocannabinol + Δ^9-tetrahydrocannabinol group, Fig. 2B; LSD test, $P < 0.05$). This confirms rapid development of tolerance to Δ^9-tetrahydrocannabinol. However, there was no rapid cross-tolerance between Δ^9-tetrahydrocannabinol and ethanol on day 2 (Δ^9-tetrahydrocannabinol + ethanol group, Fig. 2B). Indeed, these animals showed nearly identical responses to motor effects of ethanol (Fig. 2A).

3.3. Influence of a cannabinoid CB_1 receptor antagonist on the development of rapid tolerance to ethanol and Δ^9-tetrahydrocannabinol

The development of rapid tolerance to ethanol and Δ^9-tetrahydrocannabinol and the influence of a cannabinoid receptor antagonist on these responses are shown in Fig. 3A and B. For ethanol, a separate two-way ANOVA for repeated measures showed a significant effect for treatment $F(3, 27) = 6.45$, $P < 0.002$, and for day factor, $F(1, 27) = 25.33$, $P < 0.00001$. The interaction between treatment and day factors was significant (Fig. 3A; $F(3, 27) = 36.96$, $P < 0.00001$). Post hoc comparisons revealed that on day 1, mice injected with ethanol, regardless of the pretreatment (control solution or SR 141716A), showed a significant motor impairment on rota-rod performance compared to the control group (Fig. 3A; LSD test, $P < 0.05$). Pretreatment with SR 141716A did not alter the response of control animals (Fig. 3A). On day 2, the same pattern of response was observed in the control animals challenged with ethanol (control + ethanol groups); thus, both groups presented a clear motor impairment. However, animals which had received ethanol on day 1 showed significantly less motor impairment to a challenge dose of

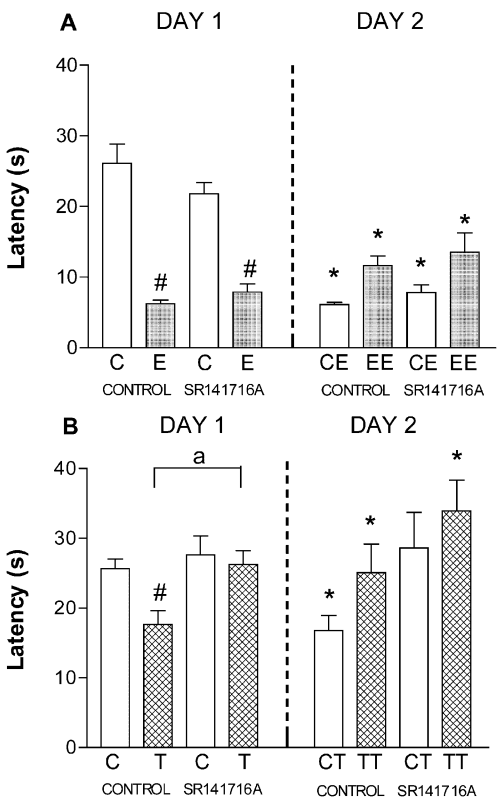

Fig. 3. Influence of cannabinoid CB_1 receptor antagonist SR 141716A (2 mg/kg i.p.) on the development of rapid tolerance to ethanol or Δ^9-tetrahydrocannabinol. (A) On day 1, pretreatment with control or SR 141716A solutions were followed by injections with control solution (C) or ethanol (E). Thirty minutes later, mice were tested on the rota-rod. On day 2, all groups were challenged with ethanol (2.25 g/kg i.p.) before testing. (B) On day 1, the same procedure as in A, except that Δ^9-tetrahydrocannabinol was injected in T groups. On day 2, all groups were challenged with Δ^9-tetrahydrocannabinol (28 mg/kg) and tested again on the rota-rod. Results shown are means ± S.E.M. of 7–12 animals per group. (#) Indicates significant difference compared to control group; (*) indicates significant difference from corresponding groups on day 1; (a) indicates significant difference compared to the respective T group (LSD test).

ethanol on day 2 when compared to the previous day (ethanol + ethanol groups, Fig. 3A; LSD test, $P < 0.05$). The extent of this tolerance to ethanol was not altered with SR 141716A pretreatment, as shown by the comparisons between the two ethanol + ethanol groups on day 2 (Fig. 3A).

Fig. 3B depicts the results of the experiment on the development of rapid tolerance to Δ^9-tetrahydrocannabinol. A two-way ANOVA for repeated measures revealed a significant effect for treatment, $F(3, 34) = 3.41$, $P < 0.028$. There was also a significant treatment × day interaction, $F(3, 34) = 3.70$, $P < 0.02$, while the day factor was not significant, $F(1, 34) = 0.67$, $P < 0.41$. Post hoc testing indicated that on day 1, control mice injected with Δ^9-tetrahydrocannabinol showed a significant motor impairment compared to the respective control animals (control + control vs. control + Δ^9-tetrahydrocannabinol group; LSD test, $P < 0.05$), while pretreatment with the cannabinoid antagonist SR 141716A significantly prevented this response in the group injected with Δ^9-tetrahydrocannabinol (control + Δ^9-tetrahydrocannabinol vs. SR 141716A + Δ^9-tetrahydrocannabinol group; LSD test, $P < 0.02$). Mice receiving Δ^9-tetrahydrocannabinol on day 1 showed significantly less motor impairment to the challenge dose of the drug on the second day of the experiment (Δ^9-tetrahydrocannabinol + Δ^9-tetrahydrocannabinol groups; LSD test, $P < 0.05$). This finding confirms the rapid development of tolerance to Δ^9-tetrahydrocannabinol. The group of mice injected with the antagonist SR 141716A before Δ^9-tetrahydrocannabinol on day 1 did not exhibit any change in rapid tolerance evaluated on day 2 since there was a significant effect of treatment with Δ^9-tetrahydrocannabinol and of pretreatment with SR 141716A, $F(1, 34) = 8.09$, $P < 0.007$, without a significant interaction between these factors (Fig. 3B).

4. Discussion

The present results clearly showed a smaller motor impairment response in mice pretreated with a single dose of Δ^9-tetrahydrocannabinol than in those pretreated with control solution. This is the first report of rapid tolerance to Δ^9-tetrahydrocannabinol, as assessed by performance on the rota-rod, being similar to that induced by ethanol using the same protocol. In addition, an asymmetrical rapid tolerance between Δ^9-tetrahydrocannabinol and ethanol was demonstrated. Thus, ethanol pretreatment produced cross-tolerance to Δ^9-tetrahydrocannabinol, while the reverse procedure failed to induce cross-tolerance. Analysis of basal motor activity just before the injection on day 2 ensured that there were no residual effects of the previous drug administration (data not shown). The 2-day design and the test doses of ethanol and Δ^9-tetrahydrocannabinol used in this study were based on previous studies (Crabbe et al., 1979; Sprague and Craigmill, 1976) and on experiments from this laboratory showing that ethanol 2.25 g/kg produced a reliable rapid tolerance measured on the rota-rod test (Barbosa and Morato, 2001). Our data confirmed an approximate potency ratio of ethanol to Δ^9-tetrahydrocannabinol similar to that found by Sprague and Craigmill (1976) in a chronic study. These results also confirm and extend the work of Pertwee et al. (1993) who reported the development of rapid tolerance to the hypothermic action of Δ^9-tetrahydrocannabinol, and the recent study of Bass and Martin (2000) showing the onset of tolerance to the antinociceptive and hypoactive effects of Δ^9-tetrahydrocannabinol within 1.5 days of injection in mice.

Although only one dose level of each drug was used in the experiment of cross-tolerance, these doses were equipotent in terms of ability to cause rapid tolerance on the rota-rod. Therefore, the improved performance of mice treated with ethanol and Δ^9-tetrahydrocannabinol can be attributed to the development of the so-called asymmetrical cross-tolerance on a rota-rod apparatus. It is noteworthy that a dose of Δ^9-tetrahydrocannabinol, which produces less acute impairment than the dose of ethanol used, produced greater rapid tolerance. This fact could be a consequence of the long duration of Δ^9-tetrahydrocannabinol action. There is convincing evidence that acute tolerance (Radlow, 1994) and chronic tolerance (LeBlanc et al., 1969) develop progressively with time. Thus, it is conceivable that development of rapid tolerance may be determined by an interaction between dose and time of exposure to the drug. This could be an alternative explanation for the apparent development of asymmetrical tolerance between ethanol and Δ^9-tetrahydrocannabinol. Our findings are at variance with earlier studies which found symmetrical chronic cross-tolerance between ethanol and Δ^9-tetrahydrocannabinol (Siemens and Doyle, 1979; Newman et al., 1974; Sprague and Craigmill, 1976), but this probably relates to methodological and species differences as well as differences in what process is being studied. Nevertheless, our data confirm other studies on rapid cross-tolerance between ethanol and some sedative/hypnotic drugs (Khanna et al., 1992).

In agreement with our previous study, the present findings indicate that the use of the rota-rod apparatus is a useful procedure for measuring tolerance to different classes of drugs (Sprague and Craigmill, 1976; Barreto et al., 1998; Barbosa and Morato, 2001). However, the magnitude of motor impairment in mice injected with ethanol was greater than that seen in mice injected with Δ^9-tetrahydrocannabinol. In this respect, it is also interesting to note that motor incoordination in response to ethanol does not seem to be mediated by a cannabinoid mechanism. Pretreatment with SR 141716A, an antagonist of the cannabinoid CB_1 receptor, did not affect the clear motor impairment effects caused by ethanol. This finding is contrary to some recent studies reported by others (Arnone et al., 1997; Colombo et al., 1998), but it must be conceded that these studies were presumably examining the

role of cannabinoid CB_1 receptors in the reinforcing mechanisms affected by ethanol.

It is noteworthy that while the pretreatment with the selected dose of SR 141716A clearly prevented the effects of Δ^9-tetrahydrocannabinol on day 1, it failed to block the rapid tolerance induced by the drug. Considering that SR 141716A blocks the development of tolerance to and dependence on Δ^9-tetrahydrocannabinol in many other models (Cook et al., 1998; Lichtman et al., 1998), this failure of the cannabinoid antagonist to block the rapid tolerance to Δ^9-tetrahydrocannabinol in mice poses a dilemma. Indeed, mice pretreated with the antagonist on the previous day showed a tendency towards an increased tolerance after the injection of Δ^9-tetrahydrocannabinol on day 2, or from another perspective, an improved performance of these animals in the rota-rod apparatus was noted. Given the hypothesis that Δ^9-tetrahydrocannabinol-induced rapid tolerance involves predominantly the regulation of the cannabinoid CB_1 receptor function, it is important to raise some possibilities for these intriguing findings. One possibility is that the inability of SR 141716A to prevent Δ^9-tetrahydrocannabinol-induced rapid tolerance may relate to the long duration of Δ^9-tetrahydrocannabinol action in this protocol. Another possibility, though more speculative, is that the observed improvement may be due to the situation of practicing the task under the influence of the drug and/or a learning factor (Bitrán and Kalant, 1991; Khanna et al., 1996) following the interaction SR 141716A $\times$ Δ^9-tetrahydrocannabinol. In this respect, a recent study (Lichtman, 2000) has shown that SR 141716A possesses memory enhancing property. Therefore, whatever the causal factors, it should be noted that SR 141716A injection on day 1 did not produce motor incoordination on its own and also did not have any residual effects on motor impairment on the second day as observed by normal basal activity for all subgroups. Lastly, the results presented herein raise the possibility that the role of cannabinoid CB_1 receptor in the development of rapid tolerance to Δ^9-tetrahydrocannabinol could be difficult to reveal in the currently used 2-day protocol as a consequence of the long duration of cannabinoid action.

Although the drug levels were not examined in the present study, it would seem unlikely that the results reported here could be attributed to differences in dispositional tolerance. Previous studies failed to show a pharmacokinetic basis for tolerance to different effects of Δ^9-tetrahydrocannabinol (Siemens and Doyle, 1979; Sprague and Craigmill, 1976; Pertwee et al., 1993) or for rapid development of tolerance to ethanol (Crabbe et al., 1979). Therefore, it is impossible to decide on the relative contribution of pharmacodynamic and pharmacokinetic factors to the rapid tolerance and cross-tolerance observed in the present study.

In conclusion, these results have shown that Δ^9-tetrahydrocannabinol induces rapid tolerance to motor incoordination in mice, similar to that elicited by ethanol, and pretreatment with a cannabinoid receptor antagonist, SR 141716A, did not prevent such tolerance. An apparently asymmetrical cross-tolerance between ethanol and Δ^9-tetrahydrocannabinol was seen, with ethanol pretreatment resulting in rapid cross-tolerance to Δ^9-tetrahydrocannabinol. The mechanisms underlying the unexpected effects of the cannabinoid CB_1 receptor antagonist on rapid tolerance to Δ^9-tetrahydrocannabinol remain to be elucidated.

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References

Adams, I.B., Martin, B.R., 1996. Cannabis: pharmacology and toxicology in animals and humans. Addiction 91, 585–614.

Arnone, M., Maruani, J., Chaperon, F., Thiébot, M., Poncelet, M., Soubrié, P., Le Fur, G., 1997. Selective inhibition of sucrose and ethanol intake by SR 141716A, an antagonist of central cannabinoid (CB_1) receptors. Psychopharmacology 132, 104–106.

Barbosa, A., Morato, G.S., 2001. Influence of neurosteroids on the development of rapid tolerance to ethanol in mice. Eur. J. Pharmacol. (in press).

Barreto, P.S., Lemos, T., Morato, G.S., 1998. NMDA-receptor antagonists block the development of rapid tolerance to ethanol in mice. Addict. Biol. 3, 55–64.

Bass, C.E., Martin, B.R., 2000. Time course for the induction and maintenance of tolerance to Δ^9-tetrahydrocannabinol in mice. Drug Alcohol Depend. 60, 113–119.

Bitrán, M., Kalant, H., 1991. Learning factor in rapid tolerance to ethanol-induced motor impairment. Pharmacol. Biochem. Behav. 39, 917–922.

Carlini, E.A., 1968. Tolerance to chronic administration of *Cannabis sativa* (marihuana) in rats. Pharmacology 1, 135–142.

Colombo, G., Agabio, R., Fa, M., Guano, L., Lobina, C., Loche, A., Reali, R., Gessa, G., 1998. Reduction of voluntary ethanol intake in ethanol-preferring sP rats by the cannabinoid antagonist SR141716. Alcohol Alcohol. 33, 126–130.

Compton, D.R., Aceto, M.D., Lowe, J., Martin, B.R., 1996. In vivo characterization of a specific cannabinoid receptor antagonist (SR 141716A): inhibition of Δ^9-tetrahydrocannabinol-induced responses and apparent agonist activity. J. Pharmacol. Exp. Ther. 277, 586–594.

Cook, S.A., Lowe, J.A., Martin, B.R., 1998. CB_1 receptor antagonist precipitates withdrawal in mice chronically exposed to Δ^9-THC. J. Pharmacol. Exp. Ther. 285, 1150–1156.

Crabbe, J.C., Rigter, H., Uijlen, J., Strijbos, C., 1979. Rapid development of tolerance to the hypothermic effect of ethanol in mice. J. Pharmacol. Exp. Ther. 208, 128–133.

Devane, W.A., Hanus, L., Breuer, A., Pertwee, R.G., Stevenson, L.A., Riffin, 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, 1946–1947.

Heishman, S.J., Arasteh, K., Stitzer, M.L., 1997. Comparative effects of alcohol and marijuana on mood, memory, and performance. Pharmacol. Biochem. Behav. 58, 93–101.

Hollister, L.E., Gillespie, H.K., 1970. Marijuana, ethanol and dextroamphetamine. Arch. Gen. Psychiatry 23, 199–203.

Kaymakcalan, S., Turker, R., Turker, M.N., 1974. Analgesic effect of Δ^9-tetrahydrocannabinol in the dog. Psychopharmacology 35, 123–128.

Khanna, J.M., Kalant, H., Weiner, J., Shah, G., 1992. Rapid tolerance and cross-tolerance as predictors of chronic tolerance and cross-tolerance. Pharmacol. Biochem. Behav. 41, 355–360.

Khanna, J.M., Chau, A., Shah, G., 1996. Characterization of the phenomenon of rapid tolerance to ethanol. Alcohol 13, 621–628.

Khanna, J.M., Shah, G., Chau, A., 1997. Effect of NMDA antagonists on rapid tolerance to ethanol under two different testing paradigms. Pharmacol. Biochem. Behav. 57, 693–697.

LeBlanc, A.E., Kalant, H., Gibbins, R.J., Berman, N.D., 1969. Acquisition and loss of tolerance to ethanol by the rat. J. Pharmacol. Exp. Ther. 168, 244–250.

Lichtman, A.H., 2000. SR 141716A enhances spatial memory as assessed in a radial-arm maze task in rats. Eur. J. Pharmacol. 404, 175–179.

Lichtman, A.H., Wiley, J.L., La Vecchia, K.L., Neviaser, S.T., Arthur, D.B., Wilson, D.M., Martin, B.R., 1998. Effects of SR 141716A after acute or chronic cannabinoid administration in dogs. Eur. J. Pharmacol. 357, 139–148.

Matsuda, L.A., Lolait, S., Browstein, M.J., Young, A.C., Bonner, T.I., 1990. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346, 561–564.

McMillan, D.E., Harris, L.S., Frankenheim, J.M., Kennedy, J.S., 1970. L-Δ^9-*trans*-tetrahydrocannabinol in pigeons: tolerance to the behavioral effects. Science 169, 501–503.

Munro, S., Thomas, K.L., Abu-Shaar, M., 1993. Molecular characterization of a peripheral receptor for cannabinoids. Nature 365, 61–65.

Newman, L.M., Lutz, M.P., Gould, M.H., Domino, E.F., 1974. Δ^9-Tetrahydrocannabinol and ethyl alcohol: evidence for cross-tolerance in the rat. Science 175, 1022–1023.

Ng, L.K., Lamprecht, F., Williams, R.B., Kopin, I.J., 1973. Δ^9-Tetrahydrocannabinol and ethanol: differential effects on sympathetic activity in differing environmental settings. Science 180, 1368–1369.

Pertwee, R.G., Stevenson, L.A., Griffin, G., 1993. Cross-tolerance between delta-9-tetrahydrocannabinol and the cannabimimetic agents, CP 55,940, WIN 55,212-2 and anandamide. Br. J. Pharmacol. 110, 1483–1490.

Radlow, R., 1994. A quantitative theory of acute tolerance to alcohol. Psychopharmacology 114, 118.

Rinaldi-Carmona, M., Barth, F., Heaulme, M., Shire, D., Calandra, B., Congy, C., Martinez, J., Neliat, G., Caput, D., Ferrara, P., Soubrie, P., Breliere, J.C., Le Fur, G., 1994. SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. FEBS Lett. 350, 240–244.

Siemens, A.J., Doyle, O.L., 1979. Cross-tolerance between Δ^9-tetrahydrocannabinol and ethanol: the role of drug disposition. Pharmacol. Biochem. Behav. 10, 49–55.

Sprague, G.L., Craigmill, A.L., 1976. Ethanol and delta-9-tetrahydrocannabinol: mechanism for cross-tolerance in mice. Pharmacol. Biochem. Behav. 5, 409–415.

Stella, N., Schweitzer, P., Piomelli, D., 1997. A second endogenous cannabinoid that modulates long-term potentiation. Nature 388, 773–778.

Takahashi, R.N., Singer, G., 1979. Self-administration of Δ^9-tetrahydrocannabinol by rats. Pharmacol. Biochem. Behav. 11, 737–740.