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Cross-Tolerance Between Δ^9-Tetrahydrocannabinol and Ethanol: The Role of Drug Disposition

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SIEMENS, A. J. AND O. L. DOYLE. *Cross-tolerance between Δ^9-tetrahydrocannabinol and ethanol: The role of drug disposition.* PHARMAC. BIOCHEM. BEHAV. 10(1) 49–55, 1979.—Acute challenge doses of Δ^9-tetrahydrocannabinol (THC), 10.1 mg/kg, administered intragastrically by gavage (IG), or ethanol, 1.24 g/kg, IP, reduced the rotarod performance of female rats by 50%. Daily treatment of the animals with THC, 10.1 mg/kg, IG, or ethanol, 4 g/kg, IG, resulted in tolerance development to the impairing effects of the challenge doses of each drug on rotarod performance. THC-tolerant animals were cross-tolerant to the challenge dose of ethanol, but ethanol-tolerant rats did not show complete cross-tolerance to the challenge dose of THC. THC-tolerant animals initially had higher blood levels of ^{14}C-THC than controls after IG drug administration. Following IV injection, the rates of ^{14}C-THC disappearance were equivalent in the latter groups. ^{14}C-THC disappearance was not altered in ethanol-tolerant animals. The rates of ethanol disappearance were not significantly modified in THC- or ethanol-tolerant animals. In conclusion, THC-tolerant female rats demonstrated cross-tolerance to ethanol as shown previously for males. Furthermore, the development of tolerance and cross-tolerance was not a function of changes in drug disappearance.

THC-ethanol cross-tolerance Rotarod performance ^{14}C-THC blood disappearance Female rats
Ethanol blood disappearance

IN VIEW OF the common use of alcohol and marihuana together in various doses and sequences, it is important to develop an understanding of how these drugs may mutually influence their actions. Tolerance develops to the depressant effects of ethanol [8, 16, 29, 31] and Δ^9-tetrahydrocannabinol (THC), the primary psychoactive constituent of marihuana [2, 5, 15, 21], in both man and animals. Several years ago, an observation [6] that the behavioral effects of ethanol were somewhat attenuated in chronic users of marihuana suggested the possibility of cross-tolerance between the two drugs. Male rats made tolerant to either ethanol or THC have been shown to exhibit symmetrical cross-tolerance to challenge doses of the opposite compound in an avoidance learning task [20]. In a subsequent study [19], male rats made tolerant to THC, as determined by lever pressing behavior, were also tolerant to a low dose of ethanol. However, other investigators [7] failed to detect tolerance to a challenge dose of ethanol in rats that were shown to be tolerant to THC using performance on a moving belt as an index of drug effect. The discrepancies in these results may be related to differences in drug doses and the types of tests used.

Neither of the latter two studies [7,19] determined if the development of tolerance to ethanol conferred tolerance to THC. In addition, the earlier experiments did not show whether the apparent cross-tolerance was functional or metabolic [8] in origin.

The experiments described below had the following goals: (a) to determine if symmetrical cross-tolerance between THC and ethanol could be observed in rats on a measure of rotarod performance, a task which is distinctly different from those used previously [7, 19, 20]; (b) to establish whether changes in drug disposition could account for any cross-tolerance observed.

Information on the development of tolerance to THC and ethanol in female animals is limited, and is based exclusively on males in relation to cross-tolerance between the two drugs. Accordingly, female rats were studied in these experiments.

METHOD

Animals and Diet

Female, Charles River CD rats, 140–165 days of age at the time of first drug treatment in all experiments, were housed individually in stainless steel, hanging cages on a 12–12 hour light-dark cycle. During the period of initial training on a rotarod, all animals had Teklad lab chow and water available ad lib. Three days before the first experimental day (see below), the animals were given free access to a balanced liquid diet (Bio-Mix No. 711, Lieber/DeCarli Diet, supplied by Bio-Serv, Inc.) to facilitate eventual pair-feeding of drug-treated and control animals. Pair-feeding was initiated on Day 27 and continued throughout the experiment. That is, each control animal was paired with a drug-treated rat on the basis of body weight, and was given the amount of diet consumed by the drug-treated animal on the previous day.

Test Procedure

Rats were trained to run on a rotarod (a 6 inch dia. rotating drum) which accelerated linearly from an initial speed of 4 rpm until the animals fell onto a platform (12 in. below). Depression of the platform activated a switch, halting the drum and a digital timer. The time on the rod varied from animal to animal, with maximum runs ending at 26–30 rpm (185–225 sec). To reduce intra-animal variability in performance from day to day, each test measurement consisted of the sum of the times of two consecutive runs. All animals were trained until performance scores varied less than 15% for each rat on 3 consecutive days.

Animals were tested immediately prior to treatment and at the time of peak drug effect; that is, at 2 hr after IG administration of THC (in olive oil solution) or olive oil (4 ml/kg) and at 30 min after IP injection of ethanol (20% w/v in saline) or saline (6.2 ml/kg). Drug effect was determined as: duration of two consecutive runs after treatment ÷ duration before treatment. Pilot studies indicated that the doses of THC and ethanol which decreased rotarod performance by 50% were 10.1 mg/kg IG and 1.24 g/kg IP, respectively. These were challenge doses used in the subsequent cross-tolerance experiments.

Animals were run on the rotarod daily throughout the experiment to maintain their performance except on the days of ethanol and THC blood disappearance studies (see below). Drugs or vehicles were administered after the daily runs except on drug test days.

Treatment and Testing Regimen

Acute treatment. The influence of challenge doses of olive oil (4 ml/kg) and THC (10.1 mg/kg) on rotarod performance was determined in all previously trained animals on Days 1 and 2, respectively. On Days 3–5, 8–15, 17–19 and 21–26 the animals were run on the rotarod without any treatment. The influence of saline (6.2 ml/kg) and ethanol (1.24 g/kg) was assessed on Days 6 and 7, respectively. The disappearance from the blood of ethanol and ^{14}C-THC was studied on Days 16 and 20, respectively.

Chronic treatment. On Day 27 animals were assigned to one of four groups of 6 rats each: Group 1 received THC, 10.1 mg/kg IG, daily; Group 2, controls for Group 1, received olive oil daily; Group 3 received ethanol at a dose of 4 g/kg, IG daily. (On test days Group 3 animals were tested with a challenge dose of ethanol (1.24 g/kg, IP). After testing, these animals received a supplemental dose of ethanol to make up 4 g/kg.) Group 4, controls for Group 3, received sucrose solution IG daily, isocaloric with the ethanol dose.

Every 3–4 days during the chronic treatment period, Groups 1 and 3 were tested for their response to challenge doses of THC or ethanol, respectively. The data were inspected on test days for apparent tolerance development. Following 12 days of THC treatment and 17 days of ethanol administration, performance scores were analyzed across days by analysis of variance for Groups 1 and 3, respectively, to determine if performance had improved significantly over time.

The day after drug tolerance was verified in the former groups, their controls were challenged with THC or ethanol, respectively—Group 2 on Day 39 and Group 4 on Day 44. Tests for cross-tolerance were performed on the following schedule: Groups 1 and 2 were injected with saline on Day 40 and with ethanol on Day 41; Groups 3 and 4 were treated with olive oil on Day 45 and with THC on Day 46.

Blood ethanol (1.24 g/kg) disappearance was studied in Groups 1 and 2 on Day 48 and in 3 and 4 on Day 53. Disappearance of ^{14}C-THC (10.1 mg/kg) from the blood after IG administration was determined on Day 51 in Groups 1 and 2, on Day 56 in Groups 3 and 4. Four days after the latter experiments ^{14}C-THC (3 mg/kg) disappearance was again studied after IV drug injection. All experimental groups continued to receive their respective daily treatments after the completion of the tolerance tests except on the days of metabolic studies.

Determination of Ethanol and ^{14}C-THC Blood Disappearance

In studies of ethanol disappearance from the blood, tail tip blood was collected serially with heparinized micropipets (50 μl) beginning at 10 min following IP injection of ethanol. Blood ethanol concentrations were determined by gas-liquid chromatography using n-propanol as internal standard [3,12].

The specific activities of ^{14}C-THC in IG and IV disappearance experiments were 4 and 3 μCi/mg, respectively. ^{14}C-THC was dissolved in olive oil (2.53 mg/ml) for IG administration, and was suspended in 2% w/v Tween 80 in saline (3 mg/ml) for IV injection via the external jugular vein [27]. The selection of the 3 mg/kg dose for IV injection was based upon experiments which showed that this amount was within a range in which the rate of THC metabolism was proportional to the dose [26]. Furthermore, this dose had previously been used in acute studies of THC-ethanol interactions [28].

In all the experiments tail tip blood was taken serially after ^{14}C-THC administration, two samples being collected at each time point. One sample (100 μl) was assayed for unchanged ^{14}C-THC by n-heptane extraction and liquid scintillation counting, while the other (50 μl) was assayed for total ^{14}C following combustion as described elsewhere [28].

Data Analysis

The organization, computation and statistical analysis of the data associated with these studies were carried out using the PROPHET System, a unique national computer resource sponsored by the NIH, and an IBM 360/67 computer. Information about PROPHET, including how to apply for access, can be obtained from the Director, Chemical/Biological Information-Handling Program, Division of Research Resources, NIH, Bethesda, Maryland 20014.

RESULTS

Acute Ethanol and THC Dose/Response Determinations

Preliminary experiments were carried out to establish the dose/response relationships between ethanol or THC and rotarod performance (Fig. 1A and B). The calculated doses of ethanol and THC which impaired performance by 50% were 1.24 g/kg and 10.1 mg/kg, respectively, based upon regression analysis of the data. These doses were used in subsequent tests for tolerance and cross-tolerance and in drug metabolism studies.

THC Tolerance and Test for Ethanol Cross-Tolerance

The development of tolerance to THC is depicted in Fig.

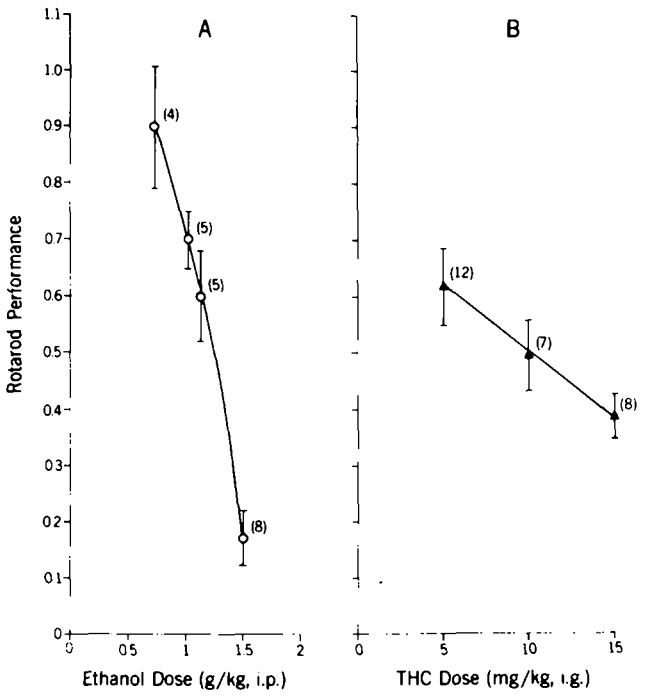

FIG. 1. The relationship between ethanol (A) or THC (B) doses and rotarod performance in female rats. Points and bars represent the means ± SEM; N's are given in parentheses.

2A. The rotarod performance of Groups 1 and 2 was significantly ($p<0.01$) impaired by THC on Day 2 relative to their respective scores obtained on Day 1 after olive oil treatment. Tolerance to the acute impairing effect of THC developed rapidly in Group 1 upon the initiation of daily THC treatment on Day 27, and was clearly established by Day 38, $F(3,15)=5.09$, $p<0.05$. Vehicle treatment did not influence performance (Group 2) throughout the study but a challenge dose of THC clearly reduced the performance score of these animals on Day 39 in comparison to their vehicle score on Day 38 ($p<0.01$). As expected, olive oil treatment did not modify the behavior of THC-tolerant rats on Day 39.

Figure 2B shows the effect of ethanol and its vehicle, saline, on the rotarod performance of the two animal groups before and after the assessment of THC tolerance in Group 1. Before chronic treatment, both groups were equivalently depressed by ethanol. However, the mean performance score of THC-tolerant animals following a challenge dose of ethanol on Day 41 was 3–4-fold higher compared to controls ($p<0.025$); thus, cross-tolerance to ethanol was observed in THC-tolerant rats.

Ethanol Tolerance and Test for THC Cross-Tolerance

As shown in Fig. 3A, ethanol depressed rotarod performance in Groups 3 and 4 on Day 7 in comparison to the respective saline effects on Day 6 ($p<0.01$). Daily ethanol administration to Group 3 began on Day 27, with tolerance being observed after 16 consecutive days of treatment, $F(4,20)=8.05$, $p<0.01$. The performance of control animals following saline injection did not change appreciably during

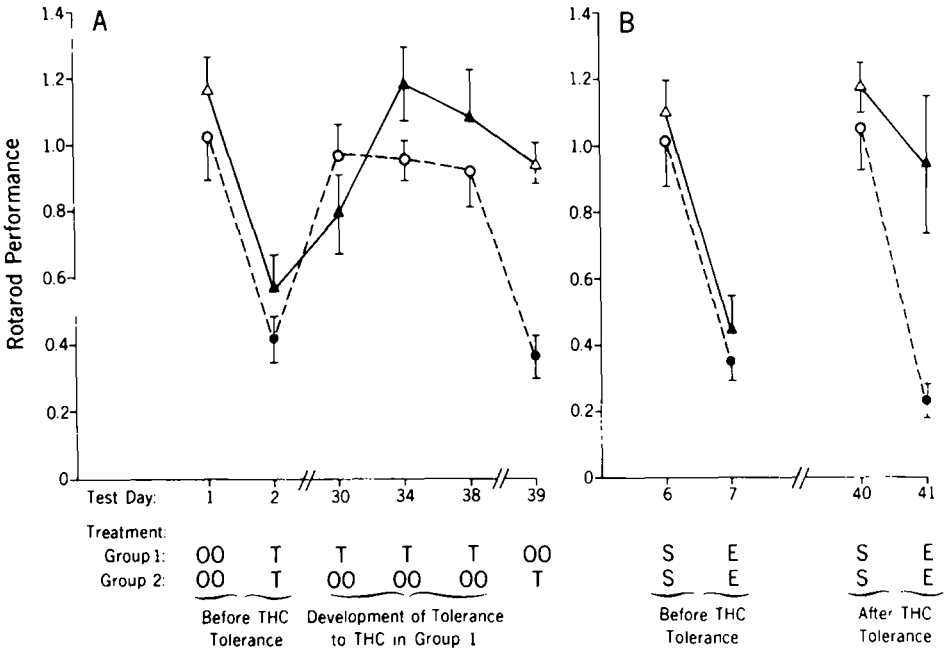

FIG. 2. (A) Development of tolerance to THC based on rotarod performance. Group 1 (triangles) received daily doses of THC beginning on Day 27 to tolerance, whereas Group 2 (circles) received olive oil daily except as specified on selected test days. On test days the groups were treated as indicated on the abscissa; solid symbols signify drug treatment, open symbols signify vehicle treatment; OO = olive oil, T = THC, N = 6/group; values are the mean ± SEM. (B) Influence of saline (S) and ethanol (E) on rotarod performance in Groups 1 and 2 before and after THC tolerance development.

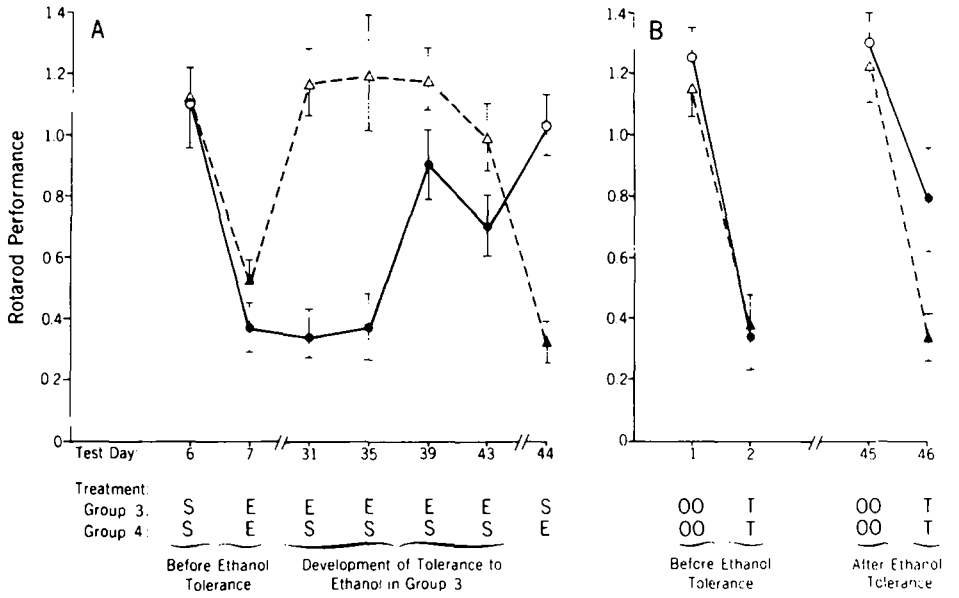

FIG. 3. (A) Development of tolerance to ethanol based on rotarod performance. Group 3 (circles) received daily doses of ethanol beginning on Day 27 to tolerance, whereas Group 4 (triangles) received sucrose daily except as specified on selected test days. On test days the groups were treated as indicated on the abscissa; solid symbols signify drug treatment, open symbols signify vehicle treatment; N = 6/group; values are the mean ± SEM. (B) Influence of olive oil and THC on rotarod performance in Groups 3 and 4 before and after ethanol tolerance development.

the study. However, their scores were significantly ($p<0.01$) reduced, as expected, under the influence of ethanol on Day 44 in comparison to vehicle treatment on Day 43.

The effects of olive oil and THC on rotarod performance in the latter two groups before and after ethanol tolerance development are illustrated in Fig. 3B. THC obviously depressed the performance of both groups in comparison to olive oil before ethanol tolerance. The mean performance score for the ethanol-tolerant rats was better than the performance of controls after THC administration on Day 46 ($p<0.05$). However, the interaction term between the periods of testing (pre- and post-ethanol tolerance), the treatment (THC and olive oil) and the two groups was not statistically significant, $F(1,10)=1.59$, $p=0.24$. Thus, tolerance to ethanol did not confer complete cross-tolerance to THC in this dosage and test system.

Drug Disappearance

The disappearance of ^{14}C-THC and ethanol from the blood was studied in all groups of animals before and after the tolerance experiments. Before the respective treatments were initiated to produce THC and ethanol tolerance, all groups of animals demonstrated equivalent blood disappearance curves (according to analysis of variance of drug concentration data) for ethanol, $F(15,100)=0.95$, $p>0.10$, for total ^{14}C, representing unchanged ^{14}C-THC plus metabolites, $F(18,120)=0.94$, $p>0.10$, and for unchanged ^{14}C-THC, $F(18,120)=0.95$, $p>0.10$.

After tolerance to THC was established in Group 1, the blood levels of both total ^{14}C and unchanged ^{14}C-THC were significantly higher from 1–8 hr following IG ^{14}C-THC administration in this group compared to their olive oil controls (Fig. 4A). Thus, THC tolerance was not related to a decrease in THC blood levels at the time of performance testing, rather the levels were higher than in controls. At 12 and 24 hr, the two groups did not differ in their blood concentrations of total ^{14}C and ^{14}C-THC. These data are not adequate to permit a reliable assessment of the rates of disappearance of radioactivity in the groups after 8 hr. However, the observation that the two groups showed equivalent blood disappearance curves for total radioactivity, $F(10,100)=0.02$, $p>0.50$, and unchanged ^{14}C-THC, $F(10,100=0.21$, $p>0.50$, following IV injection of ^{14}C-THC (Fig. 4B), suggests that THC tolerance is not due to an increase in the rate of THC metabolism.

The disappearance of ethanol from the blood of THC-tolerant rats was also clearly not different, $F(4,40)=0.30$, $p>0.50$, from that of olive oil controls (Fig. 5A). Thus, the cross-tolerance to ethanol was not of dispositional origin.

The blood ethanol concentrations in ethanol-tolerant animals (Group 3) were lower, albeit not significantly so, than in the sucrose controls at 15 and 30 min following drug injection (Fig. 5B). The slight decrease in ethanol levels was not likely sufficient to account for the ethanol tolerance which was observed at 30 min. Although the blood ethanol concentrations were significantly lower (according to t-test) in ethanol-tolerant rats than in controls at 60 and 120 min, analysis of variance showed that the groups were not significantly different over time, $F(4,40)=0.07$, $p>0.50$.

Figure 6A shows that ethanol-tolerant animals and their controls had similar blood disappearance curves for total ^{14}C, $F(6,60)=0.84$, $p>0.50$, and unchanged ^{14}C-THC, $F(6,60)=0.46$, $p>0.50$, after IG ^{14}C-THC administration. Similarly (Fig. 6B), neither the blood disappearance of total radioactivity nor of ^{14}C-THC differed, $F(10,100)=0.50$,

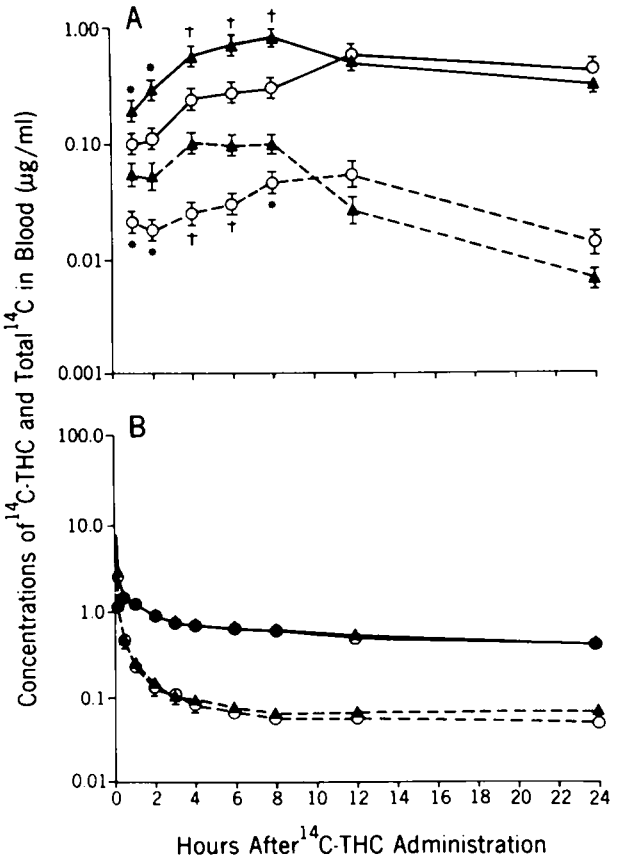

FIG. 4. Concentrations of total ^{14}C (———) and unchanged ^{14}C-THC (– – – –) in the blood of THC tolerant rats (▲) and olive oil controls (○): A—after ^{14}C-THC, 10.1 mg/kg, IG; B—after ^{14}C-THC, 3 mg/kg, IV. Points and bars represent the mean ± SEM; symbols are larger than SEM in some cases; N = 6/group; *$p<0.05$, †$p<0.005$.

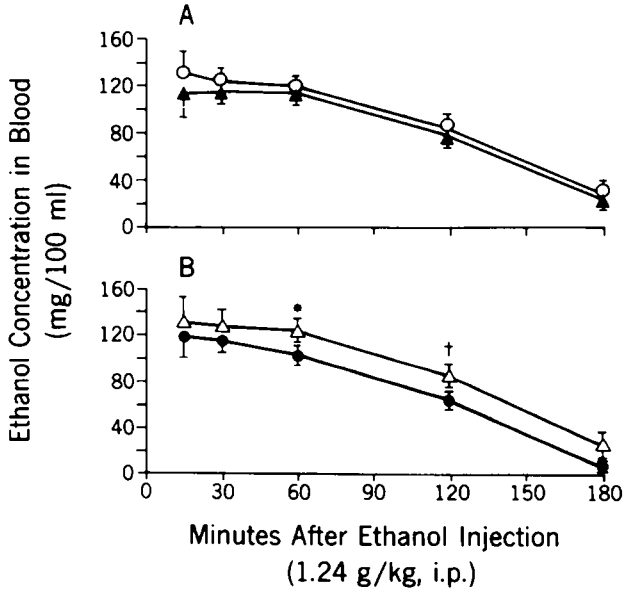

FIG. 5. Disappearance of ethanol from the blood of: A—THC-tolerant rats (▲) and olive oil controls (O); B—ethanol-tolerant rats (●) and sucrose controls (△). Points and bars represent the mean ± SEM; N = 6/group; *$p<0.05$, †$p<0.005$.

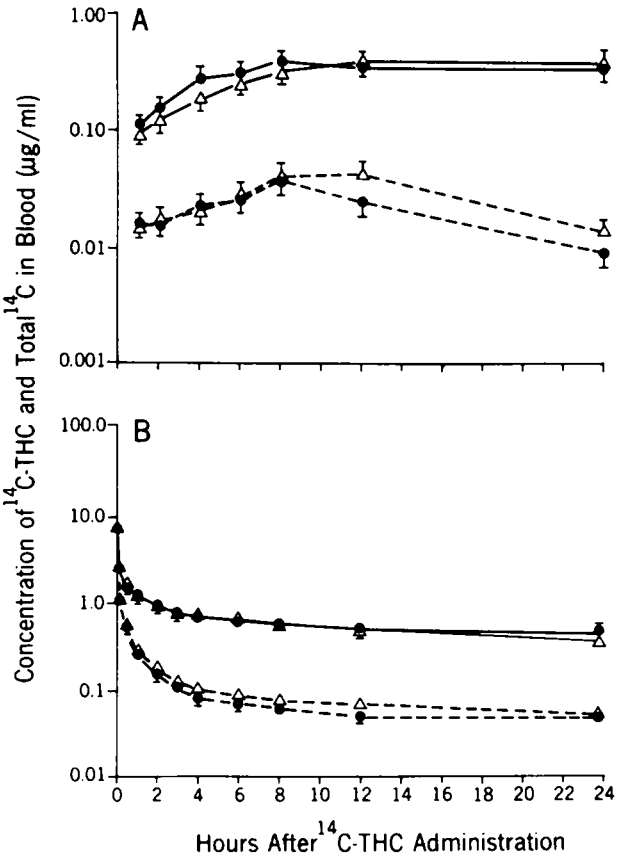

FIG. 6. Concentrations of total ^{14}C (———) and unchanged ^{14}C-THC (– – – –) in the blood of ethanol tolerant rats (●) and sucrose controls (△): A—after ^{14}C-THC, 10.1 mg/kg, IG; B—after ^{14}C-THC, 3 mg/kg, IV. Points and bars represent the mean ± SEM; N = 6/group.

$p>0.50$; $F(10,100)=1.67$, $p=0.10$, respectively, in the two groups following IV injection of ^{14}C-THC.

DISCUSSION

This study has demonstrated the development of tolerance to the impairing effects of THC and ethanol in female rats on the rotarod, a measure of motor performance. Since animals received drug treatments after daily training sessions, except on drug test days, it is not likely that the tolerance was "behaviorally augmented" [13]. It is not possible on the basis of these experiments to compare, critically, tolerance development to THC and ethanol in male and female rats. However, other studies in this laboratory have verified that the rates and magnitudes of tolerance development to THC and ethanol in male rats are similar to those in females (unpublished results).

Following the establishment of drug tolerance, the animals were assessed for possible cross-tolerance between THC and ethanol. Animals which were tolerant to THC, 10.1 mg/kg, were only minimally influenced by ethanol, 1.24 g/kg, a dose shown to be equi-effective with the THC dose in impairing rotarod performance acutely. This is consistent with previous reports that male rats, shown to be tolerant to

THC, 20 mg/kg, were cross-tolerant to challenge doses of ethanol according to two other behavioral measures, namely, conditioned avoidance ([20] and G.T. Pryor, personal communication) and lever pressing [19] tasks. Although the rotarod, avoidance and lever pressing tests all probably measure motor function to some extent, the latter two tasks also reflect more complex cognitive processes. Taken as a group, the above studies indicate that this form of THC-ethanol cross-tolerance is independent of the type of behavioral tests used. However, contrary to this conclusion, cross-tolerance to ethanol, 1.4 g/kg, could not be demonstrated by means of a moving belt test in male rats which were tolerant to THC after 17 daily doses of THC, 12 mg/kg [7]. Preliminary experiments in this laboratory have also revealed that daily treatment of female rats for 17 days with THC, 12 mg/kg/day, resulted in tolerance to THC, but not cross-tolerance to ethanol, 0.6–1.2 g/kg, on an operant behavior task. The reasons for the conflicting results are not clear but could be related to the drug doses, duration of treatment and the measures used.

The impairing effect of THC was partially, albeit not significantly, attenuated in ethanol-tolerant animals relative to controls. The same conclusion was reached in a replication of the experiment. It is possible that significant cross-tolerance to THC might be developed by the treatment of animals with higher doses of ethanol over longer periods of time resulting in a greater level of ethanol tolerance than was achieved in this study. However, recent studies (G.T. Pryor, personal communication) using shock avoidance conditioning also failed to detect significant cross-tolerance to THC in ethanol-tolerant rats. That cross-tolerance was not entirely symmetrical or reciprocal in the present study may indicate that the nature of the impairing effects of acutely equivalent doses of the two drugs were not perceived to be identical by the animals. This might be expected, among other possibilities, if these drugs act by different mechanisms, have different side effects, or if the time course of the pharmacological effects changed for either drug during chronic treatment.

Other investigators have reported apparent symmetrical cross-tolerance between the two drugs based on an avoidance task with rats [20] and rotarod activity with mice [30]. Species differences, the method of performance measurement, drug doses and duration of chronic ethanol treatment could all have contributed to these discrepancies in results. It is also important to note that in both of the latter studies, the performance scores of control groups were not apparently included in the statistical analyses of THC effects in ethanol-tolerant animals.

Daily treatment of animals with THC resulted in an elevation of the blood concentrations of unchanged ^{14}C-THC and total radioactivity (^{14}C-THC plus ^{14}C-THC metabolites) from 1–8 hours after IG ^{14}C-THC administration. Thus, THC tolerance was clearly not related to a decrease in the amount of drug available in the blood. An increase in THC blood concentrations after oral THC administration in rats has been reported previously [22], but the reasons for the higher levels are not known. The phenomenon could be a consequence of changes in THC absorption.

The almost identical disappearance characteristics of ^{14}C-THC from the blood of THC-tolerant and control rats following IV ^{14}C-THC injection suggest that chronic THC treatment did not alter the tissue distribution, plasma protein binding or the rates of metabolism and excretion of ^{14}C-THC. This observation is in agreement with earlier conclusions that THC tolerance in rats did not originate with changes in the metabolism of the drug [11,25] but rather with functional changes in the brain.

The highest concentrations of total radioactivity or ^{14}C-THC in the blood of non-tolerant rats did not occur until 8–12 hours following IG drug administration. Our previous experiments [23] revealed that peak ^{14}C concentrations in the blood of satiated rats occurred within 2–4 hours after IG ^{14}C-THC treatment in comparison to 8 hours in animals which were fasted overnight. It is possible that the liquid diet influenced the absorption of THC in these experiments. Thus, our preliminary experiments with equivalently fed female rats, showing that peak behavioral THC effects were already detected at 2 hours, indicate that the drug effects were not parallel to THC blood concentrations.

Daily treatment of the rats with THC did not alter the rate of ethanol disappearance from the blood, indicating that the observed cross-tolerance was not due to an increase in ethanol metabolism. Furthermore, chronic ethanol treatment did not change the rate of ethanol disappearance from the blood significantly, and thus ethanol tolerance was not of metabolic origin. The absence of an inductive effect of ethanol on its own metabolism is probably due to the relatively low daily dose of ethanol administered (4 g/kg/day). It is well known that ethanol metabolism may be stimulated in rats by the daily administration of 10–14 g/kg for 2–4 weeks [9, 14, 17].

Chronic treatment of rats with high doses of ethanol causes a proliferation of the hepatic smooth endoplasmic reticulum [4,24] resulting in a stimulation of the microsomal metabolism of numerous drugs [1, 18, 24]. However, the stimulation of drug metabolism is clearly not equivalent for all drugs, in some cases being nonexistent [10]. In this investigation, the relatively low doses of ethanol failed to modify the disappearance of ^{14}C-THC following IG and IV administration. Thus, the non-significant improvement in the performance of ethanol-tolerant animals following a challenge dose of THC was not due to a change in THC disposition.

In conclusion, these studies have established that tolerance to, and cross-tolerance between, ethanol and THC in female rats are not dependent on changes in the disposition of either drug. Although it is not possible to extrapolate the results of these studies to man directly, these observations support the earlier suggestion [6] that cross-tolerance between marihuana and ethanol may occur in the human.

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