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Interactions of Δ^9-Tetrahydrocannabinol with Phenobarbital, Ethanol and Chlordiazepoxide[1]

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PRYOR, G. T., F. F. LARSEN, J. D. CARR AND M. C. BRAUDE. *Interactions of Δ^9-tetrahydrocannabinol with phenobarbital, ethanol, and chlordiazepoxide in rats.* PHARMAC. BIOCHEM. BEHAV. 7(4) 331–345, 1977. – The acute, reciprocal dose-response interactions between Δ^9-tetrahydrocannabinol (Δ^9-THC; 2.5, 5.0 and 10.0 mg/kg; IG) and each of three depressants – phenobarbital (PB; 10, 20 and 40 mg/kg; IP), ethanol (ETOH; 0.5, 1.0 and 2.0 g/kg; IP), and chlordiazepoxide (CDP; 2.5, 5.0 and 10.0 mg/kg; IP) – were studied for their effects on performance of a conditioned avoidance response (CAR), photocell activity, heart rate, body temperature, and rotarod performance. Δ^9-THC impaired CAR and rotarod performance, depressed photocell activity, and decreased heart rate and body temperature. None of the three depressants significantly influenced CAR performance but they all decreased photocell activity and impaired rotarod performance at one or more doses. PB and ETOH also decreased heart rate and body temperature at the highest doses. When combined with Δ^9-THC each of the three drugs at some dose combinations caused greater depressant effects on most measures than caused by either drug alone. Only CDP did not augment the impairment of CAR performance caused by Δ^9-THC. The highest dose combinations of Δ^9-THC and each of the three drugs almost completely eliminated photocell activity and rotarod performance. The interactions were also studied after subacute treatment for six days with Δ^9-THC and/or each of the three depressants. There was clear evidence for tolerance to the effects of Δ^9-THC on all measures and this tolerance generally resulted in less interactive effects between Δ^9-THC and each of the depressants. There was also evidence for tolerance to the effects of PB and ETOH on some measures but not CDP. The reduction of effects alone or combined with Δ^9-THC could be accounted for by assuming a partial loss of potency after subacute treatment that decreased the pharmacologically effective doses of either or both interacting drugs.

Oral Δ^9-THC in rats Phenobarbital Ethanol Chlordiazepoxide Acute and subacute treatment Rotarod
Interactions between Δ^9-THC and depressants CAR Photocell activity Heart rate Body temperature

THE widespread increase in the use of marihuana in recent years has been accompanied by a corresponding increase in multiple drug use [10, 13, 31]. The consequences of the combined use of marihuana with other drugs are generally unknown in either animals or humans. Although a number of reports have appeared describing some of the interactions between cannabis or its constituents with several drugs (see [34] for selected references), no systematic attempts have been made thus far to characterize the interactions in terms of: (1) doses and blood levels of the respective drugs; (2) history of exposure to either or both drugs; or (3) the kinds of measures used to identify the interactions.

We have been engaged in such an evaluation of the possible pharmacological and metabolic interactions between a major psychoactive ingredient in marihuana – Δ^9-tetrahydrocannabinol (Δ^9-THC [1]) – and a number of other drugs from various pharmacological classes [32, 33, 34, 35, 36]. In this paper we will describe some of the preclinical behavioral and pharmacological results obtained in rats with combinations of Δ^9-THC and each of three drugs having primarily depressant properties – phenobarbital (PB), ethanol (ETOH), and chlordiazepoxide (CDP) – after acute administration and after subacute pretreatment with Δ^9-THC and/or each test drug.

There is good reason to believe that these three depressant drugs might be used concomitantly with, or in close temporal proximity to, the use of marihuana. PB is contained in 48 products listed in the 1976 Physicians' Desk Reference [30]. It is included in various formulations

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as a mild sedative prescribed for gastrointestinal disorders, pulmonary disorders such as asthma and emphasema, cardiovascular symptoms, insomnia, motion sickness, menopause, epilepsy, nervous tension, arthritic conditions, genitourinary disorders, and headache. In addition to these legitimate uses, PB along with other barbiturates are used illicitly as recreational drugs among various groups [10,13]. CDP, although apparently not yet a popular street drug, is, along with other minor tranquilizers, among the most widely prescribed drugs for symptomatic relief of nervous tension and anxiety [13]. The potentiating effects of interacting minor tranquilizers with ETOH have been recognized [3], but their effects in combination with marihuana are unknown. ETOH in the form of various alcoholic beverages is, of course, the most widely used recreational drug in the United States [3,10]. Although it was once thought that marihuana might substitute for alcohol as a recreational drug, the evidence suggests that they are frequently consumed together among users of both [13].

METHOD

Animals

Male rats of the inbred Fischer strain were used in all experiments. They were 55 to 60 days old (140 to 160 g) when received from Simonsen's Laboratories, Gilroy, California. They were housed singly in hanging wire mesh cages with food and water available at all times. The ambient temperature was 22°C and the lights in the room were turned on at 0700 hr and off at 1900 hr daily.

Apparatus

Avoidance chambers. Each avoidance chamber consisted of a 30 × 36 × 40 cm wooden box housed inside a sound-attenuated, ventilated cabinet. Scrambled, constant current 1.0 mA shock applied to 0.32 cm dia. brass rods spaced 1.27 cm apart served as the unconditioned stimulus (UCS). Downward displacement (0.16 cm) of a 1.27 cm dia. aluminum pole suspended from the center of the ceiling served as the operant response. A 7.5 W light and a 11.4 cm dia. loudspeaker provided ambient light (0.44 foot-candles measured at floor level) and an ambient 4 kHz tone (8 dB above background, that was 50 dB measured at the center of the floor using a General Radio Co. Type 1551–C sound-level meter set for A weighting). A pulsating increase in intensity above ambient that occurred at the rate of 2.5 times per sec of either the light (50 0.88 foot-candles) or the tone (to 63 dB), or the application of a low intensity, nonaversive current (120 μA) to the floor served as conditioned stimuli (CS). Twelve such chambers were interfaced with a Digital Equipment Corporation PDP 8/F computer (located in an adjoining room) that provided automatic stimulus presentation and data collection.

Photocell activity chamber. For measuring spontaneous motor activity, a single rat was placed in a black, cylindrical chamber 30 cm in dia. and 28 cm high. Six photocells positioned 1.3 cm above the floor and oriented at 60° around the periphery recorded the animal's movements on a digital counter. The chamber was housed inside a sound-attenuated, ventilated cubicle equipped with a 7.5 W light located above the center of the chamber.

Heart rate. Heart rate was measured by attaching subdural wire electrodes to both sides of the thorax under light ether anesthesia. Clip-on leads were connected through an EKG preamplifier and into a signal detector. The width of the detection window was set for each animal to exclude noise and movement artifacts. The EKG was converted to rate by a spike-interval analyzer and recorded continuously on a strip-chart recorder as interbeat intervals. The interbeat intervals were averaged visually – by drawing a best-fit line through the graph over a distance corresponding to 2 min – and converted to beats per min (bpm). Heart rate was recorded in the photocell chamber.

Body temperature. A lubricated rectal probe attached to a Yellow Springs telethermometer was used to measure body temperature to the nearest 0.1°C.

Rotarod. The rotarod was an 8.0 cm dia. wooden dowel measuring 91 cm long and suspended 46 cm above the test surface. The surface of the rotarod was covered with emory cloth to provide footing. Its rate of rotation was controlled by a variable speed motor.

Procedure

Two groups of rats were used to evaluate each drug, drug combination, and placebo condition. The first group was pretrained in a single 30 trial session to escape footshock (1.0 mA) by pulling a 20 cm pole. Each trial lasted 30 sec unless the animal responded sooner. The intertrial interval was variable and averaged 60 sec (15 to 120 sec). After pretraining this group was given three daily 60 trial sessions in which to learn to avoid the footshock by pulling a 13 cm pole in the presence of each CS that preceded the UCS by 10 sec. The CS and UCS then remained on together for 30 sec unless the trial was terminated earlier by a pole-displacement response. The three CS (tone, light, or nonaverstive footshock) were presented randomly for 20 trials each. The intertrial interval was variable, averaging 1.5 min (15 sec to 3 min). The entire 60 trial session required 2 to 2.5 hr. Response latencies and intertrial responses (ITR) were recorded on punched paper tape for processing on a CDC 6400 computer. Rats that failed to learn the escape response were discarded (with the Fischer strain, less than 5% fail to meet this criterion). Performance typically has been 80% avoidance or better to all three CS after this training phase. The test session that followed acute or subacute drug treatment was conducted in the same way as the training sessions. No appreciable loss of the avoidance response was observed after intervals of up to 14 days between training and testing in control animals.

The second group of rats was used for measuring photocell activity, heart rate, body temperature, and rotarod performance. Before receiving any drug treatment, each rat was given a 5 min pretest in the photocell activity chamber. Based on its activity score, each rat was ranked and assigned to a control or drug treatment group so that all group means were about equal. After the photocell activity pretest, each rat was given up to four practice trials to learn to stay on the rotarod for 120 sec at 6.25 rpm. Over 90% of the rats met this criterion; rotarod data from rats that failed to meet this criterion were not used. Then the wire electrodes were implanted for subsequent EKG measurement. On the test day, photocell activity was measured for 10 min. Heart rate was then monitored in the same chamber for the next 2 min. The rat was removed from the chamber and body temperature was recorded after a 1 min equilibration period. The rat was then placed on the stationary rotarod and the rotation was gradually

increased to 11 rpm. The amount of time that the rat was able to remain on the rod – up to 120 sec – was recorded.

Experimental Design

After pretraining or pretesting each rat was assigned to 1 of 25 groups (see [34]). For the next six days each rat was intubated with sesame oil (2 ml/kg) or 10 mg/kg Δ^9-THC dissolved in sesame oil, or injected IP with a selected dose of the test drug. No further training or testing occurred during this subacute treatment phase to ensure that any observed tolerance or cumulative effects of the drugs could be interpreted simply and would not be influenced by the test procedures. On the seventh day each rat was intubated with sesame oil (2 ml/kg) or 1 of 3 doses of Δ^9-THC in sesame oil (2.5, 5.0 or 10.0 mg/2 ml/kg). Ninety min later they were given an IP injection of saline (2 ml/kg) or 1 of 3 doses of the test drug in saline (doses and number of rats tested are shown in the results section for each drug). Testing began 30 min later. These times of administration before testing were chosen from preliminary experiments to provide pharmacologically active levels of each drug alone by each route of administration at the beginning of testing. Each experiment was completed in several replications with all groups being represented in each replication.

Drugs

Δ^9-THC was prepared under contract with the NIDA by the Research Triangle Institute as a 1% (w/v) solution in sesame oil. Purity was greater than 95%. When necessary this stock solution was diluted with laboratory grade sesame oil (Fisher Scientific) that also served as the placebo control. Δ^9-THC or sesame oil was administered IG using a 5 cm, 18 ga curved feeding needle (Popper and Sons, Inc.). Phenobarbital sodium (Mallinkrodt) and chlordiazepoxide HCl (Hoffmann-La Roche) were dissolved in saline; doses were calculated as the salts. Absolute ETOH (IMC Chemical Group, Inc.) was diluted to the desired dose with distilled water. The test drugs were administered IP. All drugs were given in a volume of 2 ml/kg body weight.

Data Analysis

The data for each measure were first analyzed by analysts of variance to establish the significance of any main effects or their interactions [20]. Significant F-ratios were further evaluated by *t*-tests between selected pairs of means using the pooled degrees of freedom and residual variance from the analysis of variance. In reporting the results, whenever significant comparisons between means are given the appropriate term of the analysis of variance was also significant.

RESULTS

Interactions Between Δ^9-THC and PB

Acute interactions. Figure 1 shows the acute dose-effect relationships for Δ^9-THC and PB alone and in all combinations for the five tests in this battery. Because there were no appreciable differential effects of the treatments on avoidance performance to the three CS in this and subsequent experiments, the results were combined on this measure as total conditioned avoidance responses (CAR). Also, because there were no interactive effects between Δ^9-THC and any of the drugs on intertrial responding, the results for this measure are not reported.

Δ^9-THC caused a dose-related impairment of CAR performance that was significant for doses of 5 and 10 mg/kg ($ts(213) = 2.4$ and 4.3, respectively; $ps<0.01$). There were no significant effects of PB alone on CAR performance over the range of doses tested (10–40 mg/kg, IP). However, PB interacted with Δ^9-THC to cause greater impairment than that caused by Δ^9-THC alone. This interactive effect depended on the respective doses of each drug. With 2.5 mg/kg Δ^9-THC the highest dose of PB tended to cause greater impairment than Δ^9-THC alone but the difference was not significant ($p>0.10$). However, when this dose of PB was combined with 5.0 mg/kg Δ^9-THC the enhanced impairment was marked ($t(213) = 6.2$, $p<0.01$). Finally, with 10.0 mg/kg Δ^9-THC, CAR performance was further impaired by doses of 20 and 40 mg/kg PB ($ts(213) = 1.9$ and 4.6; $ps<0.05$ and 0.01, respectively). With these dose combinations many of the animals failed to escape footshock throughout the entire 60-trial session.

Δ^9-THC decreased photocell activity as a function of dose in this experiment, but the effect was significant only for 10.0 mg/kg Δ^9-THC ($t(170) = 3.6$, $p<0.01$). The highest dose of PB alone also decreased photocell activity ($t(170) = 4.4$, $p<0.01$) by about the same amount as 10.0 mg/kg Δ^9-THC. When the two drugs were combined the depressant effect was greater than that caused by either drug alone at all doses (all $ts(170)\geq 2.4$, all $ps<0.01$ compared with comparable doses of either drug alone). The higher dose combinations of the two drugs almost completely eliminated photocell activity.

Rotarod performance was significantly impaired by the highest doses of each drug alone (10 mg/kg Δ^9-THC, $t(170) = 3.9$, $p<0.01$; 40 mg/kg PB, $t(170) = 2.0$, $p<0.05$). When combined the effect was greater impairment than that caused by either drug alone for almost all doses. The higher dose combinations of both drugs resulted in the rats not being able to maintain their balance on the rotarod even when it was stationary.

Both heart rate ($F(3,257) = 85.6$, $p<0.001$) and body temperature ($F(3,170) = 73.2$, $p<0.001$) were decreased as a function of dose of Δ^9–THC alone. Only the highest dose of PB alone caused a decrease in heart rate ($t(166) = 3.4$, $p<0.01$), whereas body temperature was also decreased more or less as a function of dose of PB ($ts(170) = 2.1$, 1.3 and 3.7; $p<0.05$, NS, and 0.01 for 10, 20, 40 mg/kg, respectively). The combinations of all doses of Δ^9-THC and PB caused greater decreases on both measures than those caused by either drug alone. The interaction appeared to be additive on these two measures.

Subacute treatment with Δ^9-THC. Figure 2 shows the acute dose-effect relationships for PB alone and in combination with 10 mg/kg Δ^9–THC compared with the effects seen after subacute pretreatment with 10 mg/kg/day Δ^9-THC for six days. The results after subacute treatment with both 10 mg/kg/day Δ^9-THC and 40 mg/kg/day PB for all seven days are also shown.

There was significant tolerance to the effects of 10 mg/kg Δ^9–THC on all measures (CAR performance : $t(338) = 3.2$; photocell activity : $t(266) = 3.9$; rotarod performance : $t(265) = 2.6$; heart rate : $t(257) = 4.8$; body temperature : $t(265) = 2.4$; all $ps<0.05$ comparing acute vs subacute treatment). This reduction of the effects of Δ^9-THC alone was accompanied by an attenuation of the depressant effects of the combination of Δ^9-THC and PB.

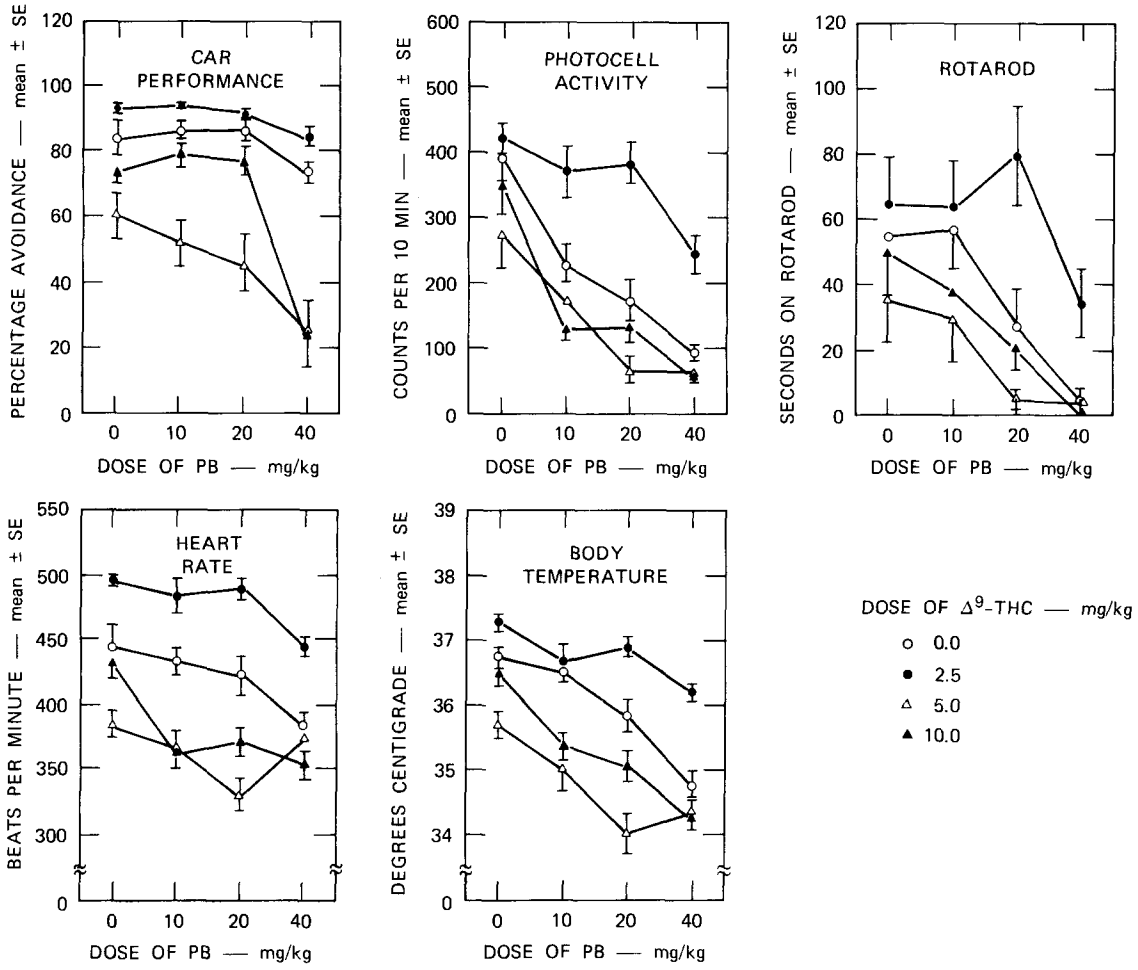

FIG. 1. Acute reciprocal dose response interactions between Δ⁹-THC and PB. There were 11 to 17 rats in each group tested for CAR performance and 10 to 12 for the other measures.

The result, in general, was an upward displacement of the dose-effect curves for PB in combination with Δ⁹-THC. However, the curves were not parallel over the entire dose range for all measures. The combination of 10 mg/kg Δ⁹-THC and 40 mg/kg PB was as effective after subacute pretreatment with Δ⁹-THC as it was when administered acutely for the effects on CAR and rotarod performance and photocell activity. Therefore, tolerance to the effects of Δ⁹-THC was not complete and sufficient residual activity remained to interact with PB at the highest doses. In fact, the effect of the combination of 10 mg/kg Δ⁹-THC and 40 mg/kg PB on CAR and rotarod performance in such Δ⁹-THC-tolerant rats was about the same as that caused by the acute combinations of 5 mg/kg Δ⁹-THC and 40 mg/kg PB (see Fig. 1).

When both drugs were given subacutely the impairing effects on CAR performance were less than after subacute treatment with Δ⁹-THC alone ($t(338) = 6.4$, $p<0.01$) suggesting tolerance to PB as well. However, such apparent tolerance to PB was not evident on the other four measures; the effects of subacute treatment with both drugs was about the same as the effects after subacute treatment with Δ⁹-THC alone.

Subacute treatment with PB. Figure 3 shows the acute dose-effect relationships for Δ⁹-THC alone and in combination with 40 mg/kg PB compared with the effects seen after subacute pretreatment with 40 mg/kg/day PB for six days. The results after subacute treatment with both Δ⁹-THC and PB are repeated for comparison.

The results after subacute treatment with both Δ⁹-THC and PB suggest that tolerance may have developed to the effects of PB on CAR performance but not on the other four measures. This conclusion was partially supported by the results shown in Fig. 3. There were no significant effects of 40 mg/kg PB alone on CAR performance when given acutely or after subacute treatment. However, the acute impairing effect of the combination of PB and Δ⁹-THC on CAR performance was eliminated after subacute treatment with PB; there were no significant differences between any dose of Δ⁹-THC alone and the same dose combined with 40 mg/kg PB after subacute treatment with PB (all $ps>0.1$). On the other hand, the effects of PB alone and in combination with Δ⁹-THC on the other four measures were not attenuated by subacute treatment with PB to as great an extent as the effects on CAR performance. In most cases the magnitude of the depression caused by the combination of PB and Δ⁹-THC was almost as great on these measures after subacute treatment with PB as it was when they were combined acutely. However, there was significant attenuation of effect for the lower doses of

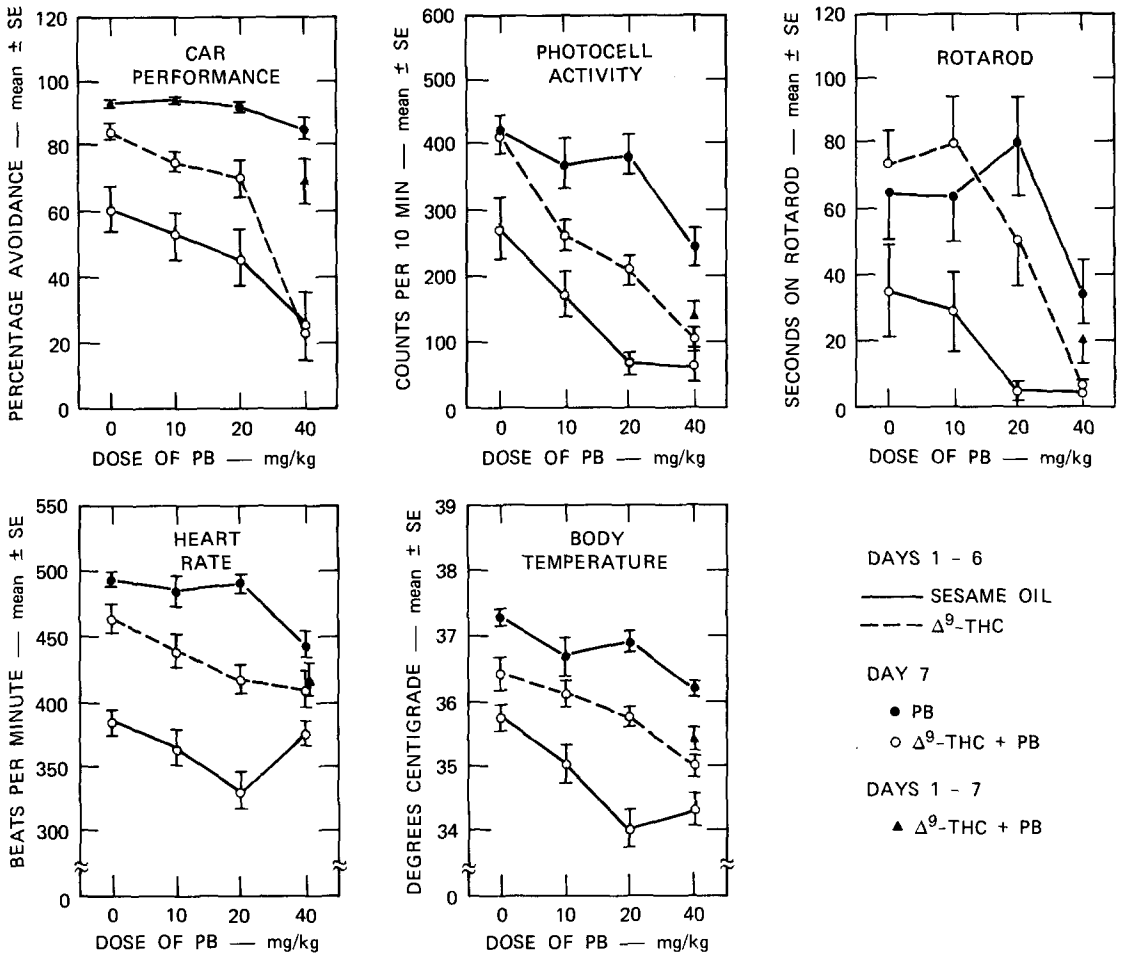

FIG. 2. Interactions between Δ⁹-THC and PB after subacute treatment with 10 mg/kg/day Δ⁹-THC for six days. There were 11 to 17 rats in each group tested for CAR performance and 10 to 12 for the other measures.

Δ⁹-THC on photocell activity (2.5 mg/kg, $t(266) = 2.6$, $p<0.01$) and body temperature (2.5 and 5.0 mg/kg, $ts(265)\geq 2.0$, $ps<0.05$) suggesting a slight tolerance to the effects of PB on these measures as well.

Interactions Between Δ⁹-THC and ETOH

Acute interactions. Figure 4 shows the acute dose-effect relationships for Δ⁹-THC and ETOH alone and in all combination for the five tests in this battery.

The acute effects of Δ⁹-THC alone were essentially the same as in the previous experiment – dose-related impairment of CAR and rotarod performance, depression of photocell activity, bradycardia, and hypothermia.

The highest dose of ETOH (2 g/kg) alone tended to impair CAR performance, but the effect was not significant ($p>0.1$). However, this dose of ETOH in combination with all doses of Δ⁹-THC caused greater impairment than that caused by Δ⁹-THC alone (all $ts(260)\geq 3.8$, all $ps\leq 0.01$). None of the lower doses of ETOH was effective in this regard when combined with Δ⁹-THC.

There were no significant effects of 0.5 or 1.0 g/kg ETOH on photocell activity, whereas 2.0 g/kg ETOH markedly depressed activity ($t(185) = 6.2$, $p<0.01$) and to a greater extent than that caused by 10 mg/kg Δ⁹-THC ($t(185) = 3.1$, $p<0.01$). When combined with Δ⁹-THC, ETOH caused a dose-related depression of photocell activity with all doses of Δ⁹-THC and the depression was greater than that caused by either drug alone in all cases. Photocell activity was almost completely eliminated when 2.0 g/kg ETOH was combined with even the lowest dose of Δ⁹-THC.

The effects of ETOH alone and in combination with Δ⁹-THC on rotarod performance were similar to those on photocell activity. Although there was a trend toward a dose-related impairment of rotarod performance caused by ETOH, the effect was significant for the highest dose only ($t(186) = 6.3$, $p<0.01$). However, the impairment caused by this dose was severe and few animals were able to remain on the rotarod for more than a few seconds. Further impairment of rotarod performance was seen when ETOH was combined with Δ⁹-THC; a dose of 2 g/kg ETOH when combined with the lowest dose of Δ⁹-THC was sufficient to prevent the animals from balancing on the stationary rotarod.

The highest dose of ETOH also decreased heart rate ($t(185) = 4.3$, $p<0.01$) and body temperature ($t(186) = 8.3$, $p<0.01$), but none of the lower doses were effective on

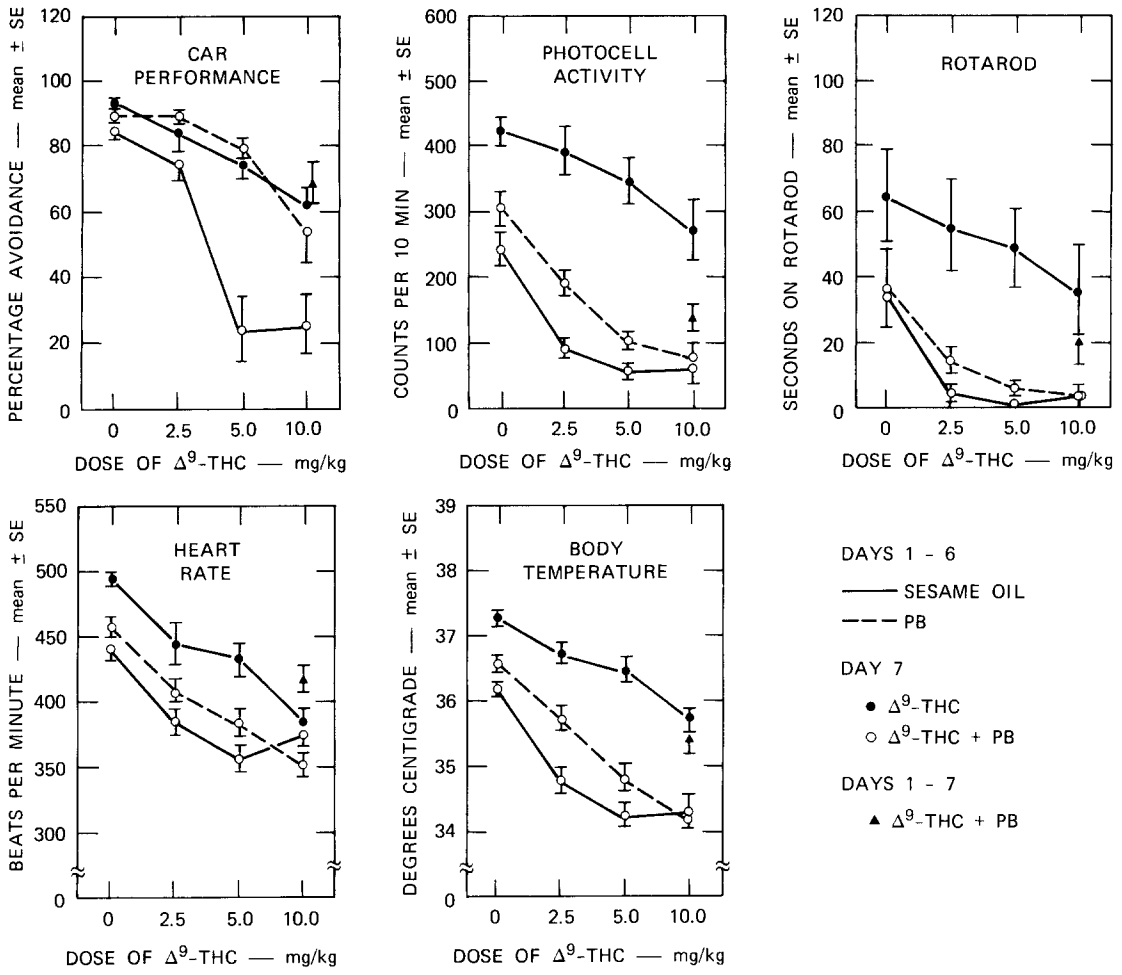

FIG. 3. Interactions between Δ⁹-THC and PB after subacute treatment with 40 mg/kg/day PB for six days. There were 11 to 17 rats in each group tested for CAR performance and 10 to 12 for the other measures.

these measures. This dose of ETOH appeared to have an additive bradycardic effect with the lower doses of Δ⁹-THC, but this trend was reversed with 10 mg/kg Δ⁹-THC. On the other hand, ETOH caused a generally dose-related potentiation of hypothermia when combined with all doses of Δ⁹-THC (all $ts(186) \geq 2.0$, all $ps \leq 0.05$).

Subacute treatment with Δ⁹-THC. Figure 5 shows the acute dose-effect relationships for ETOH alone and in combination with 10 mg/kg Δ⁹-THC compared with the effects seen after subacute pretreatment with 10 mg/kg/day Δ⁹-THC for six days. The results after subacute treatment with both 10 mg/kg/day Δ⁹-THC and 2 g/kg/day ETOH for all seven days are also shown.

Tolerance to the effects of Δ⁹-THC on all measures was again evident (all $ps < 0.05$ comparing acute vs subacute treatment). The tolerance to the effects on CAR performance did not appear to be as complete in this as in the last experiment. However, inspection of the raw data revealed that two rats were mainly responsible for this difference.

The combined effects of ETOH and Δ⁹-THC were significantly less after subacute pretreatment with Δ⁹-THC than they were when given together acutely (all $ts(415) \geq 2.3$, all $ps < 0.05$). The dose-response curve was displaced upwards reflecting the tolerance to Δ⁹-THC. However, the highest dose of ETOH was still able to potentiate the impairment caused by Δ⁹-THC ($t(415) = 2.6$, $p < 0.01$). The effect of the combination of 10 mg/kg Δ⁹-THC and 2 g/kg ETOH in such Δ⁹-THC-tolerant rats ($\bar{X} = 59.1\%$ CAR) was about the same as the effect of the combination of 5 mg/kg Δ⁹-THC and 2 g/kg ETOH in nontolerant rats ($\bar{X} = 53.1\%$ CAR). The CAR performance of rats treated subacutely with both Δ⁹-THC and ETOH was about the same as the CAR performance of rats treated subacutely with Δ⁹-THC alone. Thus, the tolerance to the effects of Δ⁹-THC on this measure was not accompanied by any apparent additional tolerance to the effects of ETOH.

Subacute treatment with Δ⁹-THC attenuated the depressant effects of Δ⁹-THC on photocell activity and its interaction with ETOH on this measure. There was no difference between the photocell activity of the group treated subacutely with 10 mg/kg Δ⁹-THC and sesame oil-treated controls. ETOH caused a linear, dose-related depression of photocell activity in such Δ⁹-THC-tolerant rats (all $ts(290) \geq 2.3$, all $ps < 0.05$) but the effect was less at

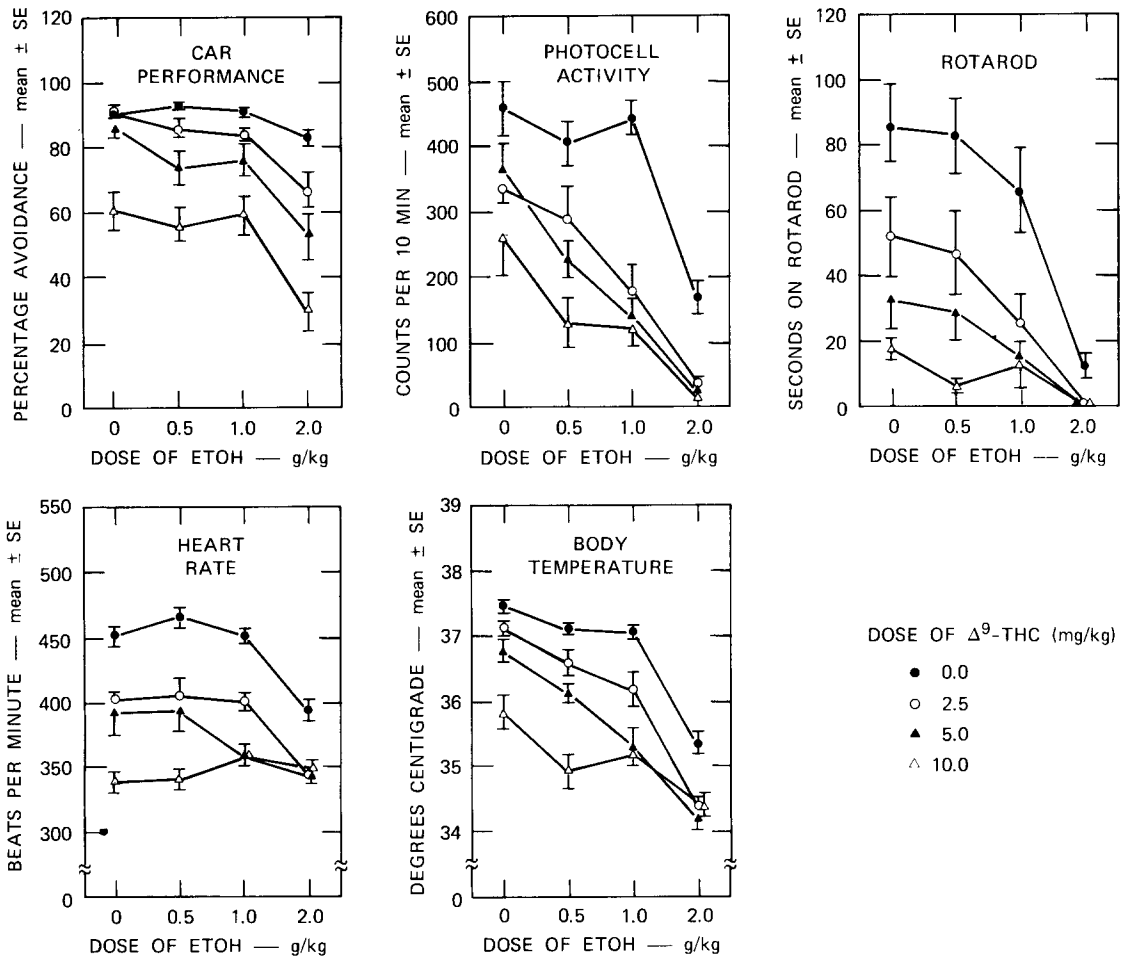

FIG. 4. Acute reciprocal dose response interactions between Δ⁹-THC and ETOH. There were 16 to 19 rats in each group tested for CAR performance and 12 to 13 for the other measures.

all doses than when the two drugs were combined acutely. Even so, the combination of Δ⁹-THC with the highest dose of ETOH in such Δ⁹-THC-tolerant rats caused almost complete suppression of photocell activity, the effect being only slightly less than that caused by their acute administration ($t(290) = 1.7$, $p<0.1$). The effects on photocell activity after subacute treatment with both Δ⁹-THC and ETOH were about the same as after subacute treatment with Δ⁹-THC alone, again suggesting no tolerance to the effects of ETOH.

The results for rotarod performance were similar to those just described for photocell activity. There was tolerance to the effect of Δ⁹-THC alone on this measure and this tolerance was reflected in an attenuation of the interactive effects of Δ⁹-THC with ETOH. In fact, the effect of ETOH on rotarod performance in such Δ⁹-THC-tolerant rats was not significantly different from that caused by ETOH alone for any dose except 1.0 g/kg ($t(292) = 2.7$, $p<0.01$).

The interactive effects of Δ⁹-THC and ETOH on heart rate and body temperature were also attenuated after subacute pretreatment with Δ⁹-THC. This reduction of effect was significant for the two lower doses of ETOH for heart rate ($ts(291) = 7.6$ and 2.6, $ps<0.01$) and body temperature ($ts(292) = 6.4$ and 2.1, $ps<0.05$). There was no further significant attenuation caused by subacute treatment with both Δ⁹-THC and ETOH.

Subacute treatment with ETOH. Figure 6 shows the results after subacute treatment with 2 g/kg/day ETOH for six days. The results after subacute pretreatment with both Δ⁹-THC and ETOH are repeated for comparison.

Whereas there was clear tolerance to the effects of Δ⁹-THC on CAR performance and its interaction with ETOH on this measure, there was no indication of a comparable tolerance to ETOH. The impairment caused by the combination of Δ⁹-THC and ETOH was the same at all doses after subacute treatment with ETOH as with sesame oil (all $ps>0.1$).

However, there was significant tolerance to the depressant effect of 2 g/kg ETOH on photocell activity ($t(290) = 4.3$, $p<0.01$, acute vs subacute) but it was not sufficient to offset the combined effects of both Δ⁹-THC and ETOH. For all doses of Δ⁹-THC the depression of photocell activity was essentially the same after subacute treatment with ETOH as sesame oil (all $ps>0.1$).

Partial tolerance to the effect of ETOH alone on rotarod performance was also seen ($t(292)) = 2.1$, $p<0.05$), but, again, it was not sufficient to prevent significantly the marked impairment caused by the combination of Δ⁹-THC and ETOH (all $ps>0.1$).

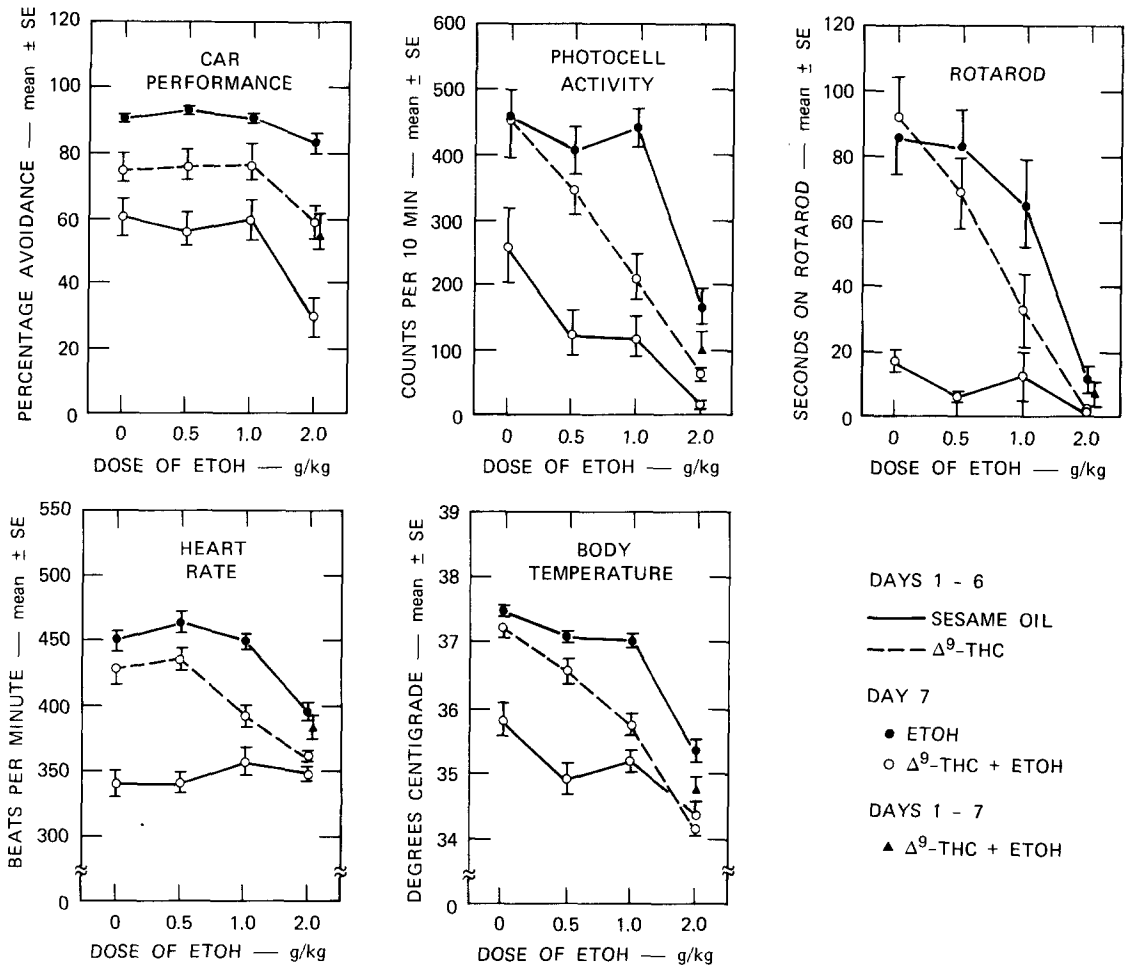

FIG. 5. Interactions between Δ⁹-THC and ETOH after subacute treatment with 10 mg/kg/day Δ⁹-THC for six days. There were 16 to 19 rats in each group tested for CAR performance and 12 to 13 for the other measures.

The bradycardia caused by acute administration of 2 g/kg ETOH was effectively eliminated after subacute treatment with ETOH, whereas there was only partial tolerance to the effect on body temperature. The combined effect of 2.5 mg/kg Δ⁹-THC and 2.0 g/kg ETOH was less in such ETOH-pretreated rats than after acute treatment on heart rate ($t(291) = 2.5$, $p<0.05$) and body temperature ($t(292) = 2.0$, $p<0.05$), but not with the higher doses of Δ⁹-THC.

Interactions Between Δ⁹-THC and CDP

Acute interactions. Figure 7 shows the acute dose-effect relationships for Δ⁹-THC and CDP alone and in all combinations for the five tests in this battery.

The results for Δ⁹-THC alone were essentially the same as in the previous two experiments. Minor exceptions were the apparent increases (not significant) in photocell activity caused by 5.0 mg/kg Δ⁹-THC and the lack of effect of the lower doses on body temperature. Since there were only 5 rats per group in this experiment we attribute these discrepancies to small sample fluctuations.

CDP did not significantly affect CAR performance over this dose range (2.5 to 10.0 mg/kg) nor did it interact with Δ⁹-THC on this measure. However, CDP caused a dose-related decrease in photocell activity ($F(3,66) = 6.5$, $p<0.001$) and rotarod performance ($F(3,66) = 7.9$, $p<0.001$). The combinations of Δ⁹-THC and CDP caused further decreases in photocell activity and rotarod performance. The interactive effects on these two measures appeared to be additive and depended on the respective doses of the two drugs. In contrast to the effects on CAR performance, the higher doses of both drugs caused almost complete suppression of photocell activity and impairment of rotarod performance when combined.

CDP alone did not significantly affect heart rate or body temperature, although a nonsignificant decrease in the latter measure was seen for the highest dose (10.0 mg/kg). However, CDP augmented the bradycardia and hypothermia caused by Δ⁹-THC as a function of dose of CDP. In all cases the effects were greater with the combinations of Δ⁹-THC and CDP than they were with either drug alone.

Subacute treatment with Δ⁹-THC. Figure 8 shows the results after subacute pretreatment with 10 mg/kg/day Δ⁹-THC for six days along with the effects of subacute treatment with both 10 mg/kg/day Δ⁹-THC and 5.0 mg/kg/day CDP for all seven days.

Again, clear tolerance to the effects of Δ⁹-THC on all

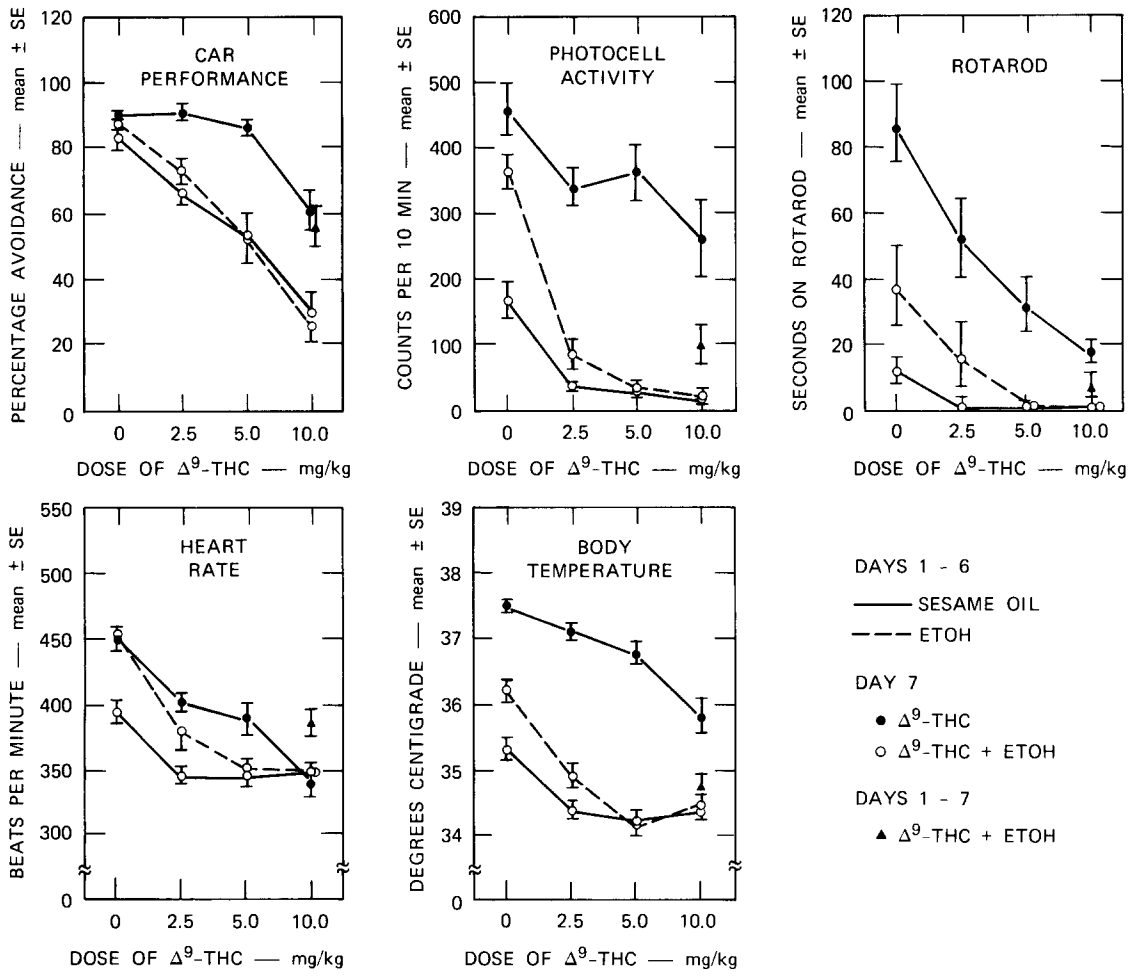

FIG. 6. Interactions between Δ⁹-THC and ETOH after subacute treatment with 2 g/kg/day ETOH for six days. There were 16 to 19 rats in each group tested for CAR performance and 12 to 13 for the other measures.

measures was seen. CDP did not alter the effects of Δ⁹-THC on CAR performance in such Δ⁹-THC-tolerant rats.

The tolerance that developed to the depressant effect of Δ⁹-THC on photocell activity resulted in a reduction of the depression caused by the combination of Δ⁹-THC and CDP. For all doses of CDP the effects caused by its combination with Δ⁹-THC in such Δ⁹-THC-tolerant rats were intermediate between those of CDP alone and their acute combination. There were no significant differences between the groups treated with CDP alone or in combination with Δ⁹-THC after subacute treatment with Δ⁹-THC (all $ts(101) \leq 1.6$, all $ps > 0.1$).

Tolerance developed to the effects of Δ⁹-THC on rotarod performance ($t(102) = 2.9$, $p < 0.01$), but it was not sufficient to offset appreciably the marked impairment caused by the combination of Δ⁹-THC and CDP. There were no significant differences between acute treatment with any dose of CDP and Δ⁹-THC compared with the same dose combinations after subacute treatment with Δ⁹-THC (all $ts(102) \leq 1.1$, all $ps > 0.1$).

Pretreatment with Δ⁹-THC caused an upward shift in the dose-effect curves for the effects of the combination of Δ⁹—THC and CDP on both heart rate and body temperature. This attenuation was significant for all doses of CDP for heart rate (all $ts(102) \geq 2.0$, all $ps < 0.05$) but not for body temperature (all $ps > 0.05$). For both measures the combined effects of Δ⁹-THC and CDP were still greater than those caused by Δ⁹-THC alone in such Δ⁹-THC-tolerant rats.

The results after subacute treatment with both Δ⁹-THC and CDP were not significantly different from the results after subacute treatment with Δ⁹-THC alone.

Subacute treatment with CDP. Figure 9 shows the results after subacute treatment with 5 mg/kg/day CDP. The results after subacute treatment with both Δ⁹-THC and CDP are repeated for comparison.

There was no evidence for tolerance to any of the effects of CDP alone or in combination with Δ⁹-THC on any of the measures. The only difference caused by subacute treatment with CDP was a greater impairment of rotarod performance caused by CDP alone compared with acute treatment ($t(102) = 2.6$, $p < 0.01$).

DISCUSSION

The results of these experiments provide a set of basic,

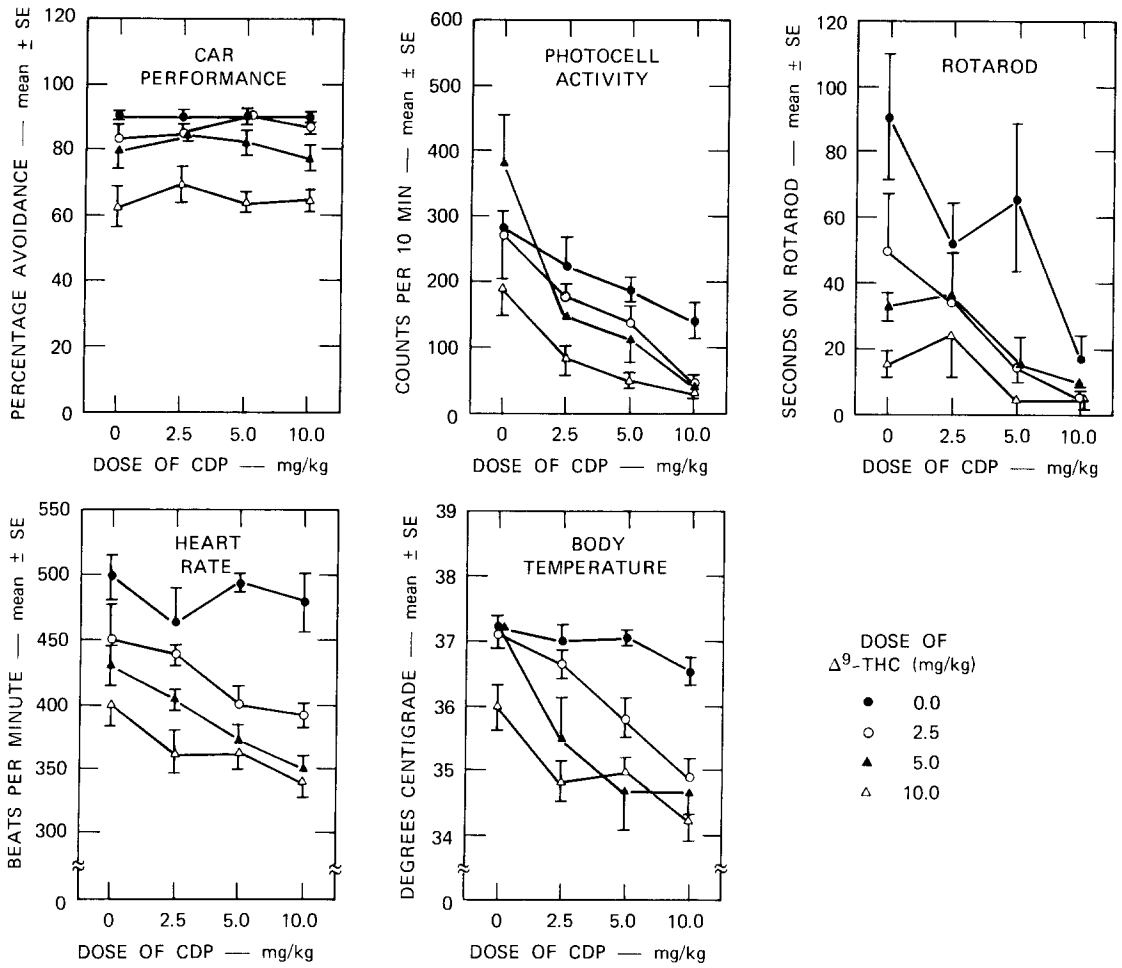

FIG. 7. Acute reciprocal dose response interactions between Δ⁹-THC and CDP. There were 14 to 17 rats in each group tested for CAR performance and 4 to 6 for the other measures.

comparable data about the interactions between Δ⁹-THC and each of the three CNS depressants studied. In general, the three drugs – PB, ETOH and CDP – interacted with Δ⁹-THC in a similar way; namely, to mutually enhance any depressent properties of either Δ⁹-THC or the test drug. However, the three drugs also differed among each other in several ways: (1) the dose-response combination patterns with Δ⁹-THC; (2) the relationship between the magnitude of the interaction and the response being measured; and (3) the influence of subacute pretreatment with Δ⁹-THC and/or the test drug.

Acute Interactions

None of the three test drugs caused any appreciable effects on CAR performance when administered alone over the dose ranges employed, whereas Δ⁹-THC caused a dose-related impairment. When given in combination with Δ⁹-THC both PB and ETOH augmented the impairment of CAR performance caused by Δ⁹-THC, whereas there was no interaction between Δ⁹-THC and CDP on this measure. Moreover, there was a difference between PB and ETOH as they interacted with Δ⁹-THC as a function of the dose of each drug. For PB it appeared that a critical dose of either PB or Δ⁹-THC was sufficient to cause an enhanced response. Thus, with 5 mg/kg Δ⁹-THC and 40 mg/kg PB the effect was much greater impairment than that caused by either drug alone, but none of the lower doses of PB had this effect, whereas with 10 mg/kg Δ⁹-THC, PB caused an additional dose-related impairment. Viewed in another way a dose of 2.5 mg/kg Δ⁹-THC was without effect alone or when combined with even the highest dose of PB, but a dose of 5 mg/kg Δ⁹-THC was clearly synergistic with 40 mg/kg PB. On the other hand, the dose of ETOH appeared critical for its interaction with Δ⁹-THC on this measure. ETOH in doses up to 1 g/kg did not influence the impairment of CAR performance caused by any dose of Δ⁹-THC, whereas 2 g/kg ETOH augmented the impairment caused by all doses of Δ⁹-THC. Thus, although both PB and ETOH had similar qualitative interactive effects with Δ⁹-THC on CAR performance, they differed from each other in terms of dose relationships.

The lack of any interactive effect of CDP with Δ⁹-THC on CAR performance was not due to the use of pharmacologically inactive doses of CDP. Clear depressant and interactive effects of these doses of CDP were evident on the other measures in this test battery. Although higher doses of CDP might also affect CAR performance alone and interact with Δ⁹-THC in this regard, the present results demonstrate a clear difference between CDP and either PB

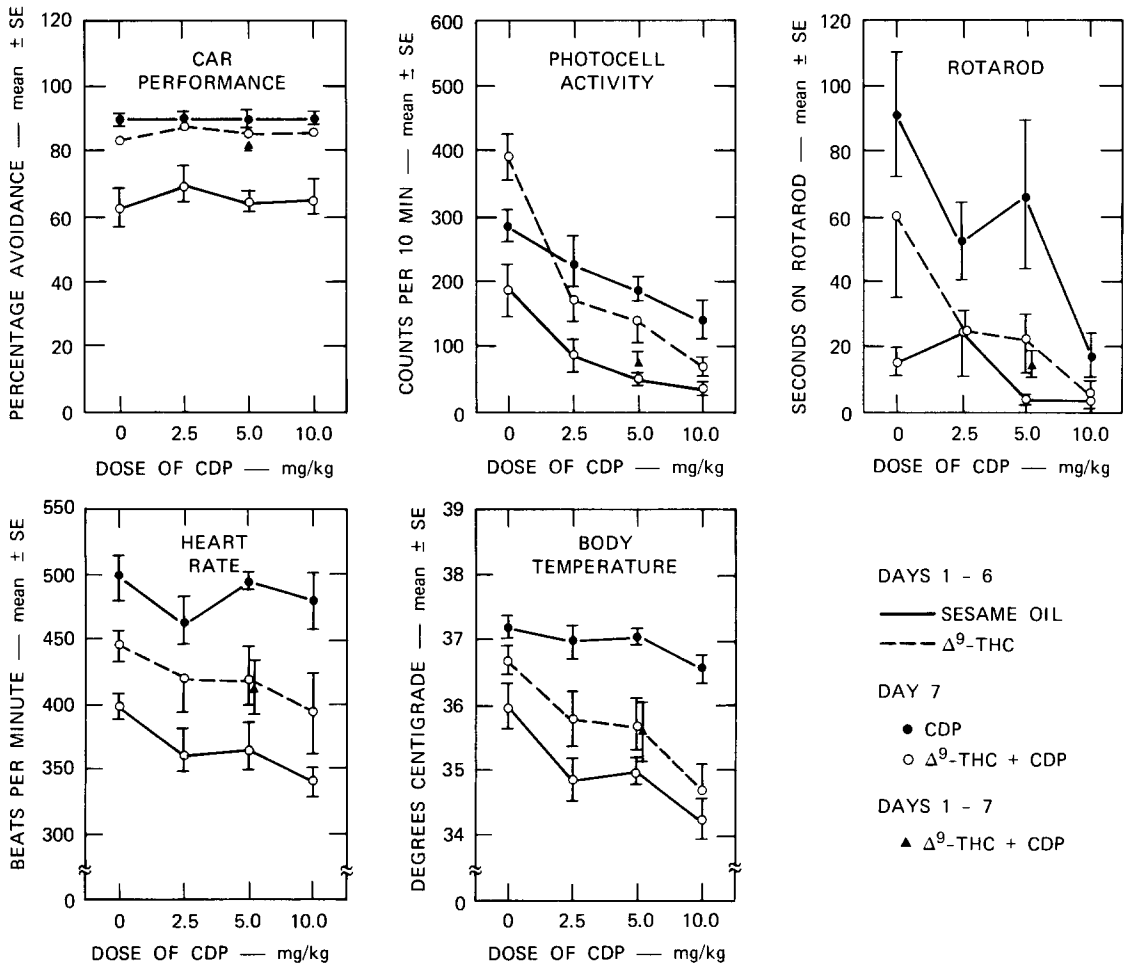

FIG. 8. Interactions between Δ⁹-THC and CDP after subacute treatment with 10 mg/kg/day Δ⁹-THC for six days. There were 14 to 17 rats in each group tested for CAR performance and 4 to 6 for the other measures.

or ETOH in their profile of effects of a given dose and their interactions with Δ⁹-THC.

Photocell activity provided another measure that demonstrated both similarities and differences among the three drugs. On this measure PB and ETOH were quite similar in that only the highest doses of each drug caused a decrease in activity, whereas both interacted with Δ⁹-THC to mutually augment the depression caused primarily by Δ⁹-THC. This interactive effect of PB and ETOH with Δ⁹-THC was dose related and was apparent with all doses of Δ⁹-THC. Thus, the interactions appeared to be more than additive for these drug combinations. In contrast, CDP caused a dose-related reduction in photocell activity and this effect was approximately additive with that of Δ⁹-THC. The higher doses of all three drugs when combined with higher doses of Δ⁹-THC caused almost complete suppression of photocell activity. That this effect did not represent complete behavioral collapse and/or anesthesia was evidenced by the fact that CAR performance was relatively intact at many of these same dose combinations. Thus, the animals were capable of performing a coordinated motor response signalled and/or motivated by suitable sensory stimuli.

The effect of all three test drugs alone and in combination with Δ⁹-THC were similar on rotarod performance. Each drug was capable of significantly impairing performance when administered alone at a sufficient dose. When combined with Δ⁹-THC they all augmented the Δ⁹-THC-induced impairment as a function of dose. Performance deteriorated rapidly with increasing dose combinations. However, the effect appeared to depend more on the dose of Δ⁹-THC than the dose of the test drug. At the highest dose combinations the dose response functions converged to maximum impairment since most of the rats did not remain on the rotarod even when it was stationary, a result similar to that seen for photocell activity but different than for CAR performance.

Both heart rate and body temperature were decreased as a function of dose of Δ⁹-THC, whereas only the highest doses of PB (40 mg/kg) and ETOH (2 g/kg) were effective in this regard and no dose of CDP had any significant influence on these measures. However, all three drugs at some doses augmented the bradycardia and hypothermia caused by Δ⁹-THC. There were only minor differences among the three drugs in the magnitude and the shapes of the dose-response functions for these interactive effects. For heart rate the interactive effects seemed to converge to that caused by the highest dose of Δ⁹-THC alone. That is,

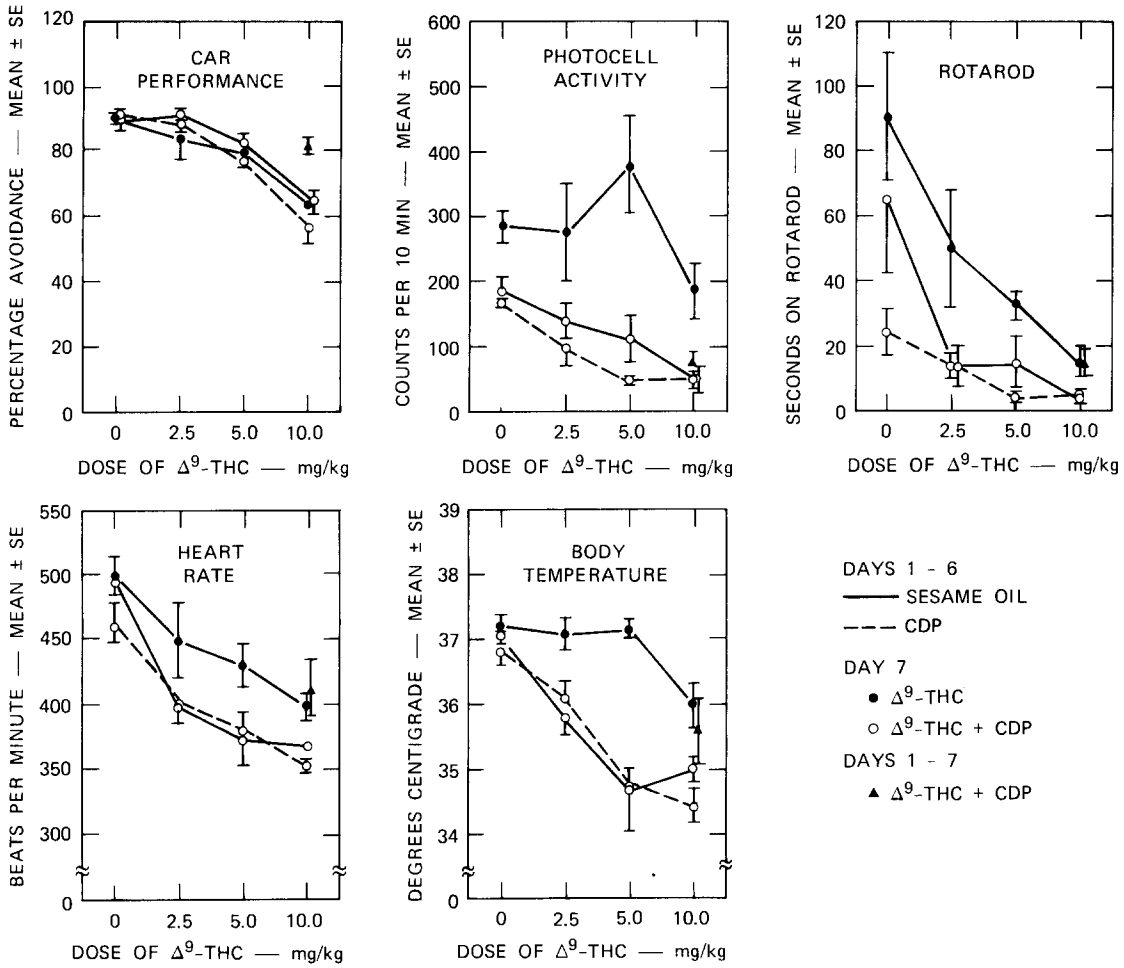

FIG. 9. Interactions between Δ9-THC and CDP after subacute treatment with 5 mg/kg/day CDP for six days. There were 14 to 17 rats in each group tested for CAR performance and 4 to 6 for the other measures.

with successively higher doses of PB, ETOH or CDP that were ineffective alone the decreases in heart rate caused by the lower doses of Δ9-THC were augmented to that caused by 10 mg/kg Δ9-THC. However, this maximum effect was not generally exceeded. The apparent decrease caused by CDP when combined with 10 mg/kg Δ9-THC may have been an artifact of the small sample sizes in that experiment because the effect of 10 mg/kg Δ9-THC alone was somewhat less than in the other two experiments. In all three experiments heart rate did not decrease below about 350 bpm (from control values of about 450–500 bpm) with any dose combinations. Thus, a lower limit may exist on this measure that, short of death, activates compensatory cardiovascular mechanisms to offset further decreases. Similarly, body temperture was decreased as a function of the combined doses of Δ9-THC and each test drugs to converge on a value of about 34.5°C (from control values of about 37.4°C). This value was reached with dose combinations that were less than the maximums used for all three drugs.

Subacute Δ9-THC

Apparent tolerance developed to the effects of 10 mg/kg Δ9-THC on all five measures in the test battery used in these experiments. This result is in agreement with previous studies in our laboratory for these tests [32, 33, 34, 35, 36]. Because the subacute treatment phase of each experiment was independent of the testing, it is likely that this diminution of effect represents primarily pharmacological tolerance rather than behavioral tolerance as some [2,22] have claimed (however, see [17]). The tolerance was not absolute and depended on the specific measure. The diminution of effect on CAR and rotarod performance and photocell activity was such that, generally, there were no differences between sesame oil-treated controls and rats treated subacutely with Δ9-THC. On the other hand there was only partial attenuation of the bradycardic and hypothermic effects. Further evidence for the conclusion that the tolerance to Δ9-THC was only partial was provided by the results obtained by combining Δ9-THC and the test drugs in such Δ9-THC pretreated rats. In some instances, depending on dose, the combined effects of the combinations were as great after subacute treatment with Δ9-THC as they were in sesame oil-treated controls. On the other hand, the partial tolerance that was evident for Δ9-THC was also reflected in an attenuation of the effects of the combination of Δ9-THC and the test drugs for many doses. The degree of attenuation of the interactive effects depended on the particular drug, the dose of the drug, and

the test measure. For both ETOH and CDP the tolerance to the effects of Δ⁹-THC on CAR performance resulted simply in an upward displacement of the dose-response function. For PB there was also an upward displacement of the curve over part of the dose range, but the highest dose of PB (40 mg/kg) in combination with 10 mg/kg Δ⁹-THC caused as much impairment in CAR performance after subacute treatment with Δ⁹-THC as after their acute combination. Since the acute combination of 5 mg/kg Δ⁹-THC and 40 mg/kg PB caused a similar degree of impairment this result suggests that the residual activity of Δ⁹-THC in such Δ⁹-THC-pretreated rats was equivalent to about 5 mg/kg Δ⁹-THC in drug-naive rats, at least in terms of its interaction with PB on this measure.

Tolerance to the depressant effect of Δ⁹-THC on photocell activity was such that there was no longer any depression after subacute pretreatment with Δ⁹-THC. However, the depression caused by combinations of Δ⁹-THC with PB, ETOH and CDP was greater than would be expected if the tolerance was complete and absolute. Thus, although PB in doses of 10 and 20 mg/kg was ineffective alone, in combination with Δ⁹-THC, it significantly decreased photocell activity in such Δ⁹-THC-pretreated rats, albeit to a significantly lesser extent than in drug-naive rats. Similar results were found for ETOH and CDP. Indeed, the depression caused by combinations of the highest doses of PB (40 mg/kg), ETOH (2 g/kg), and CDP (10 mg/kg) in combination with Δ⁹-THC was almost as great in Δ⁹-THC-tolerant rats as in rats pretreated subacutely with sesame oil. However, these doses of each drug also had significant depressant effects alone and caused almost complete suppression of photocell activity when combined acutely with as little as 2.5 mg/kg Δ⁹-THC.

The interactive effects of PB, ETOH and CDP with Δ⁹-THC on the other three measures – rotarod performance, heart rate and body temperature – were also partially attenuated by subacute pretreatment with Δ⁹-THC reflecting the tolerance to Δ⁹-THC. There were some differences among the three drugs in this regard, but none that could not be accounted for by considering the particular doses and the presumed residual activity of Δ⁹-THC.

Subacute Depressants

Tolerance did not develop as much to PB, ETOH and CDP as to Δ⁹-THC with the dosing schedule used. With few exceptions, in those instances where there was apparent tolerance to the effects of the test drug alone, it was usually not sufficient to offset the augmented depressant effects of the combination of Δ⁹-THC and the test drug. One exception was seen with PB for which CAR performance was restored to that caused by Δ⁹-THC alone after subacute treatment with PB. This result was interesting because acute administration of 40 mg/kg PB alone did not significantly impair CAR performance and, therefore, there was no indication that tolerance to PB on this measure had occurred. Only when the marked impairing effects of the combination of Δ⁹-THC and PB were considered was the tolerance to PB made manifest. Similarly, there was no significant attenuation of the depression of photocell activity or the hypothermia caused by PB alone after subacute treatment with PB, but the combined effects of the combinations of PB with the lower doses of Δ⁹-THC were less. Little or no tolerance to the effects of PB alone or in combination with Δ⁹-THC on rotarod performance or heart rate was evident.

Tolerance to many of the effects of ETOH have been demonstrated previously (e.g., [18,19]). However, the results of the present experiments showed that such tolerance is not absolute, at least with the dosing schedule used. Thus, there was no tolerance to the combined effects of ETOH and Δ⁹-THC on CAR performance. On the other hand, there was clear tolerance to the depressant effect of ETOH alone on photocell activity, but it was not sufficient to offset the marked depression caused by the combination of ETOH and Δ⁹-THC, whereas the tolerance to the effects on rotarod performance, heart rate and body temperature appeared to lessen slightly these interactive effects with the lowest doses of Δ⁹-THC.

Unlike Δ⁹-THC, PB and ETOH there was no evidence for tolerance to any of the effects of CDP alone or in combination with Δ⁹-THC. Indeed, if anything, the effects of CDP were slightly greater after subacute treatment with CDP than when administered acutely. However, this apparent cumulative effect of CDP was only significant for rotarod performance in these experiments.

Subacute Δ⁹-THC Plus Depressants

With one exception subacute treatment with both Δ⁹-THC and each of the test drugs did not cause any greater attenuation of effects than what could be accounted for by the tolerance to Δ⁹-THC alone. The exception was seen for the combined effects of Δ⁹-THC and PB on CAR performance, where the tolerance to PB also contributed to a further lessening of the impairing effect. A similar combined tolerance was not apparent for the other measures or for ETOH where tolerance was clearly demonstrated on some. This implies that sufficient residual activity of either or both drugs was still present in combination to mask any tolerance to the ETOH. More extensive dose-response studies would likely reveal the combined tolerance.

General Comments

The interactions of cannabis with the barbiturates and ETOH have received some experimental attention already, whereas the interactions with such drugs as CDP have gone virtually unexamined. However, even the information available for the barbiturates and ETOH is limited in terms of any comprehensive assessment of their interactions with cannabis. Of the papers we reviewed dealing with the barbiturates, the measures used were sleeping time [5, 7, 8, 9, 16, 28, 29, 37, 38, 39, 40], locomotor activity [16], conditioned avoidance [25], toxicity [24], and anticonvulsant activity [4, 7, 15], all in rats and mice. Human subjects were used in the two other papers. Dalton *et al.* [6] found only additive effects of secobarbital and smoked Δ⁹-THC on a number of performance measures, whereas Johnstone *et al.* [12] reported that pentobarbital was synergistic with the tachycardia caused by Δ⁹-THC. The latter investigators also reported that 5 of 7 subjects developed hallucinations and anxiety and 4 of 7 failed to complete the experiment because of severe psychologic effects when given pentobarbital (100 mg/70 kg, IV) and Δ⁹-THC (up to 134 μg/kg, IV) together. These are large doses of Δ⁹-THC, but only 2 of 10 subjects who received Δ⁹-THC alone in doses up to 210 μg/kg dropped out of an earlier study because of anxiety [21].

Of the 7 papers we reviewed dealing with ETOH, the experimental subjects were rats and mice in 6, and the measures used were sleeping time [29], conditioned avoidance [25,26], rotarod performance [41], the ability to remain on a moving belt [14], and serum dopamine β-hydroxylase activity [27]. Interestingly, the earliest report dealing with the interactions between marihuana and ETOH was with humans [23] in which the investigators found additive effects on several measures.

In the only paper we found that dealt marginally with the interactions between Δ^9-THC and CDP, Goldberg *et al.* [11] reported asymmetrical dissociation in a state-dependent learning paradigm with mice.

Although a few relevant papers may have been missed, our review was sufficient to convince us of the lack of systematic information about the interactions between marihuana and these three representative depressant drugs. Therefore, the experiments reported here were designed to provide such a set of preclinical pharmacological information that could serve as a framework for future studies. In fact, our results did not reveal any unexpected interactions between Δ^9-THC and these three drugs that might not have been predicted from a knowledge of the individual properties of each and the limited information cited above suggesting mutual potentiation. All three drugs have CNS depressant properties in sufficient dosage and would be expected, in general, to augment any depressant properties of Δ^9-THC. Indeed, perhaps the most striking feature of these experiments was the almost complete conformity of the results across a number of tests with what would be expected. The magnitude of the interactions was a function of the respective doses of the two interacting drugs, although it appeared that the critical drug dosages depended on the particular drug combination. This general finding also extended to the interactions seen after subacute treatment with Δ^9-THC. Thus, although there was tolerance to the effects of Δ^9-THC, it was only partial and the residual activity acted like a lower dose in interacting with each of the three drugs. This latter result was interesting in that in many instances the apparent tolerance to Δ^9-THC appeared to be complete on many measures. Only when the drugs were combined in such Δ^9-THC-pretreated rats was the partial nature of tolerance revealed.

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