Reduction of the Behavioral Effects of Δ⁹-Tetrahydrocannabinol by Hyperbaric Pressure[1,2]

J. MICHAEL WALSH[3,4] AND LINDA S. BURCH[3]

Behavioral Sciences Department, Naval Medical Research Institute, Bethesda, MD 20014

WALSH, J. M. AND LINDA S. BURCH. *Reduction of the behavioral effects of Δ⁹-tetrahydrocannabinol by hyperbaric pressure.* PHARMAC. BIOCHEM. BEHAV. 7(2) 111–116, 1977. — Delta⁹-tetrahydrocannabinol in doses of 0.5, 1.0, 2.0 and 4.0 mg/kg was administered to rats under normal (1 ATA) and increased (3, 5 and 7 times normal) atmospheric pressure. Behavior was maintained by a food-reinforced differential-reinforcement-of-low-rate (DRL) schedule. Dose-dependent decrements in performance were observed at the 1 ATA conditions, in which response rates increased and the well-established temporal discrimination disintegrated. Under the elevated pressure conditions, however, a reversal of behavioral toxicity occurred during which performance improved as a function of pressure. The behavioral disruptions occurring at the 5- and 7-ATA pressures were minimal compared with those occurring at 1 ATA under equivalent doses of the drug. The present experiment has demonstrated that delta⁹-tetrahydrocannabinol produces significant behavioral changes at 1 ATA pressure, but when atmospheric pressure is increased the drug effects are reduced.

Operant   Δ⁹-Tetrahydrocannabinol   DRL   Hyperbaric   Marijuana

NUMEROUS reports [1,14] have dealt with the combined effects of pharmacological agents and chemical compounds under increased barometric pressures, but few have focused on the behavioral aspects of this combination. The hyperbaric environment — to which the undersea diver and tunnel worker is exposed — is known to cause detrimental behavioral changes by itself. Thus, the increasing amount of military, industrial, and sport diving creates particular concern about the behavioral toxicity of drugs in the hyperbaric environment.

The respiration of ordinary air under increased pressure is known to result in a pharmacological effect called nitrogen narcosis. This effect is characterized by an euphoric, intoxicated state, which is often accompanied by lack of judgment and motor coordination, and sometimes by amnesia. The term nitrogen narcosis was derived from the theory which suggested that the phenomenon resulted from the increased partial pressures of nitrogen. New research [15], however, has indicated that the increased partial pressure of oxygen plays a synergistic role.

Recently a series of behavioral investigations concerned primarily with the environmental conditions encountered by the ordinary scuba diver (i.e., 2–10 ATA, breathing compressed air) were undertaken to determine the behavioral toxicity of pharmacological agents in the hyperbaric environment. The results of these studies have indicated that the behavioral effects of drugs change under pressure and that these changes are not predictable from the characteristics of the drug at sea level [17, 18, 19].

The present study is part of a continuing program to investigate the potential problems of self-medication and abuse of drugs by the diving population. The purpose of the present evaluation was to examine the behavioral effects of Δ⁹-tetrahydrocannabinol (THC) under increased pressures of air. Delta⁹-THC is the main psychoactive ingredient in marijuana, a commonly abused drug, which has been implicated in several scuba accidents [3]. Because marijuana is known to produce significant changes in sensation and perception under normal atmospheric conditions, it was hypothesized that THC would be extremely toxic to behavior under increased pressures of air.

METHOD

Animals

Four male Sprague-Dawley rats (Nmri:O[SD]CV stock) maintained at 80% free-feeding weight (250–300 g) were used. Animals were housed in individual cages with continuous access to water. Food intake was limited to 14 g/day to maintain weight; animals not receiving a full food allotment during the experimental session received the remainder in the home cage immediately after the session.

Apparatus

The experimental apparatus was a standard operant rodent cage (21.6 × 24.1 × 20.3 cm) containing two BRS/LVE rodent levers mounted on either side of a food cup centered on the front wall of the cage. In this experiment, only the left lever was used. A minimum force of 10 g on the lever was required to make an electrical switch closure, which was recorded as a response. Food pellets (45 mg Noyes) were delivered into the food cup as reinforcements by a pellet feeder mounted behind the intelligence panel. A houselight was mounted near the top-center of the intelligence panel and a 12-VDC stimulus light was mounted above each lever. During most baseline sessions the entire apparatus was mounted inside a sound-attenuated housing. Programming and recording were accomplished by a system of solid-state logic modules (BRS/LVE).

All of the pressure sessions and some baseline sessions were made with the apparatus mounted in a dry hyperbaric chamber. The chamber is cylindrical with a volume of approximately 170 liters and can withstand internal pressure of 30.3 ATA. The chamber is penetrated with several threaded openings for pressure-fitted connectors to the gas supply and the various solid-state instrumentation associated with the rat box. Compressed air (i.e., 78.1% N_2, 20.9% O_2, 0.9% Ar, 0.03% CO_2, 0.003% rare gases) was supplied to the chamber via a series of pressure-reducing valves and regulators. A system of heating and cooling coils around the inside chamber wall thermostatically maintained chamber temperature at 25 + 2°C.

Procedure

Food-reinforced lever pressing was established by successive approximation. Eventually, the rats were required to space their responses on a differential reinforcement-of-low-rate schedule (DRL), which was gradually lengthened and limited to the terminal specification of DRL 18 sec with a 6-sec limited hold. On this schedule a pellet was produced by a response on the lever that followed the preceding response by at least 18 sec, but not more than 24 sec. Responses that fell outside this time window (i.e., <18 sec, or >24 sec) went unreinforced and served only to reset the timing mechanism.

A series of 12 event counters were programmed to record interresponse times over the 24-sec interval in bins of 2 sec each. A 13th counter recorded each response that followed the response preceding it by more than 24 sec. Responses in each bin were accumulated over any one session, which provided a temporal distribution of the animals' responses within the interval.

Drug and Pressure Sessions

All of the pressure exposures in this experiment were multi-staged, where the animal was placed in the chamber for a 20-min baseline period, then compressed at a rate of approximately 1 atm/min (14.7 psi/min) with 20-min testing stops at 3, then 5, then 7 ATA. Decompression followed 7-ATA schedules, which are detailed elsewhere [16].

Delta⁹-THC, which had been dissolved in dehydrated alcohol to the appropriate concentrations, was obtained from the National Institute of Mental Health (Batch Nos. SSC-69056, SSC-79803); it was administered in doses of 0.5, 1.0, 2.0 and 4.0 mg/kg. These prepared solutions allowed the volume of each administration to be kept relatively constant at 0.1 ± 0.05 ml/100 gms of body weight. The drug solution was administered orally 60 min prior to a dive and 80 min prior to a drug control session. Occasionally equivalent volumes of dehydrated alcohol were given as placebo controls.

The four doses of the drug were each evaluated within a 4-week block. The blocks consisted of one experimental manipulation per week as shown below.

Week	Experimental Condition
1	pressure (placebo)
2	drug under pressure
3	drug control
4	drug under pressure

Performance under pressure was evaluated during the first week in each block. In the second and fourth weeks a single dose of THC was evaluated under increased pressures of air. In the third week the same dose was evaluated at 1 ATA. Daily baseline sessions were conducted between each experimental manipulation. The block (dose) order was semirandom, wherein all animals received the drug by block in the following order: 1.0, 2.0, 0.5, 4.0 mg/kg.
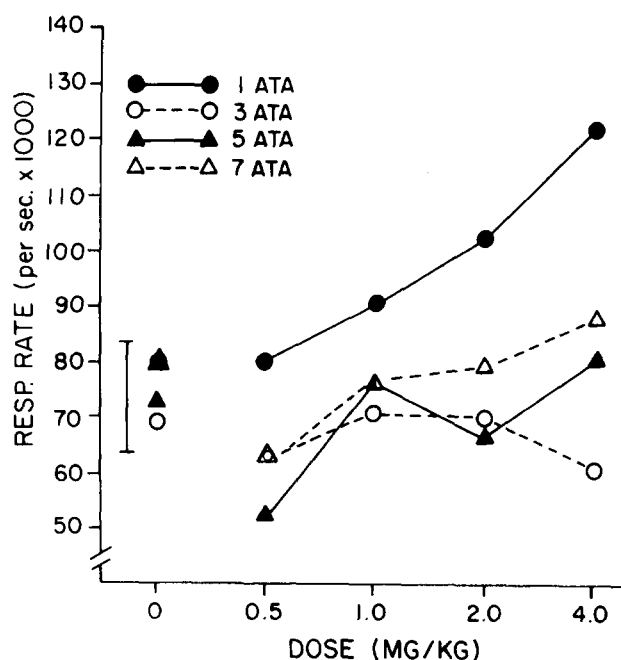

FIG. 1. Mean response rates of four animals as a function of dosage at each of four pressures. The range of baseline response rates is shown by brackets.
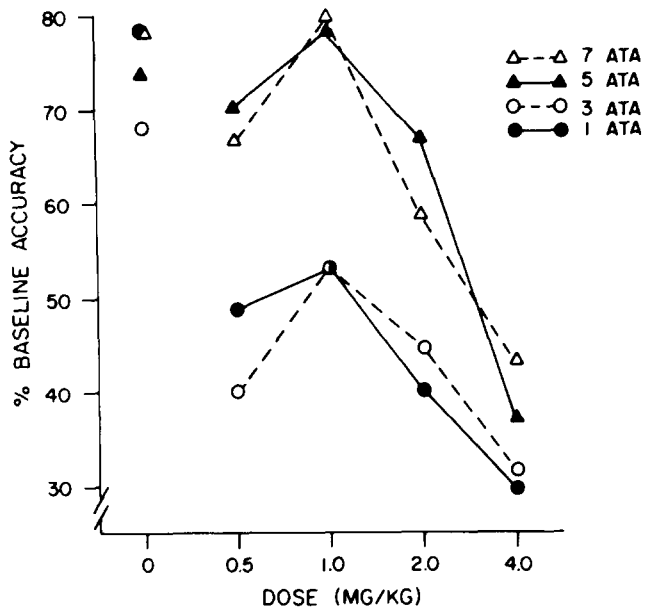
FIG. 2. Mean percents of baseline accuracy of four animals as a function of dosage at each of four pressures.

RESULTS

For all animals, doses in excess of 0.5 mg/kg resulted in disruption of performance at 1 ATA. Rate of responding in all cases increased and accuracy of the timing response was reduced. Under increased pressures of air, however, these changes were attenuated such that performance was generally better for all doses at 5 and 7 ATA.

Figure 1 shows changes in behavior as a function of the four pressure levels across the dose range. Under the placebo condition performance remained relatively constant across all pressure levels. Derived dose-response curves indicated that at 1 ATA response rates increased as a function of dose. At 3, 5 and 7 ATA rates decreased at the lower doses, but remained within control range at high-dose values. There was a trend toward increasing response rates with higher doses, but rates were nowhere near the magnitude of change observed at a 1-ATA level. An analysis of variance indicated a significant effect for pressure $F(3,24) = 3.9, p < 0.02$.

Figure 2 illustrates the changes in the accuracy of performance for the four experimental pressures at each dose level. When animals were administered the placebo, accuracy (percent of correct responses) was essentially equivalent across the four pressure levels. At the 0.5 mg/kg
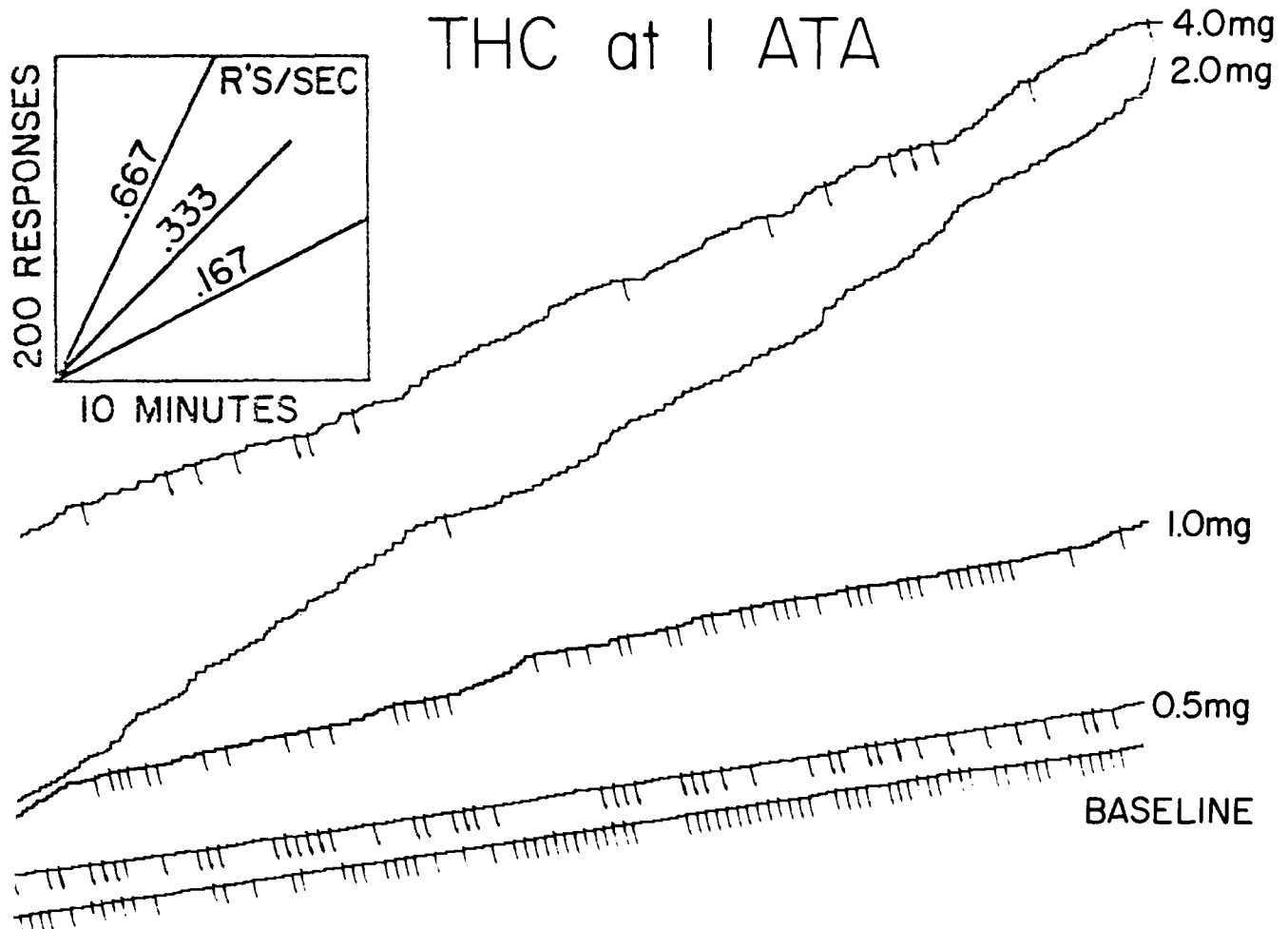

FIG. 3. Cumulative response records of one subject for baseline and four doses of Δ⁹-THC under normal atmospheric conditions. Deflections of the response pen indicate food reinforcements.
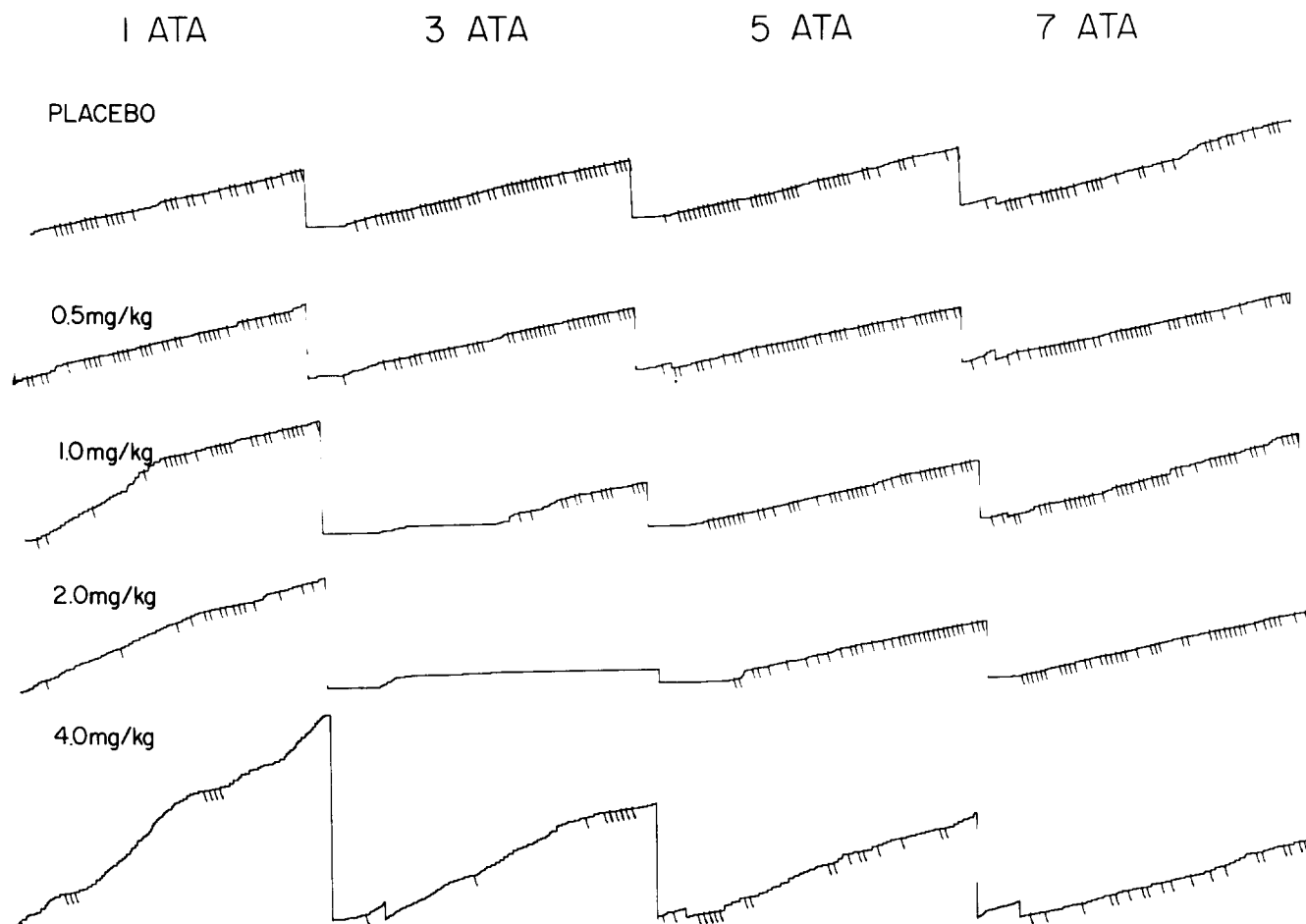
I ATA   3 ATA   5 ATA   7 ATA

PLACEBO

0.5mg/kg

1.0mg/kg

2.0mg/kg

4.0mg/kg

FIG. 4. Cumulative response records of one subject for placebo and four doses of Δ^9-THC at each of the four pressures investigated. Food reinforcements are indicated by deflections of the response pen.

dose, accuracy dropped sharply at the 1- and 3-ATA levels and continued to decrease as a function of dose. At 5 and 7 ATA, accuracy was significantly greater than at 1 or 3 ATA across the entire dose range. At these pressures (5 and 7 ATA) accuracy remained essentially unchanged at the two lower dose values and decrements in accuracy were observed only at the 2.0 and 4.0 mg/kg doses. A significant pressure effect was noted in the analysis of performance accuracy $F(3,24) = 3.42$, $p < 0.03$, as well as for drug $F(3,24) = 10.4, p < 0.01$.

Figure 3 illustrates the cumulative records for a single animal under the five dose conditions at 1 ATA. These records show drug-induced changes in behavior in the time frame roughly equivalent to the 3-, 5- and 7-ATA portion of a pressure exposure. The slope of the 0.5 mg/kg record does not differ from the baseline record, but increases in response rate are evidenced by changes in slope at the 1.0 mg/kg dose level, and at 2.0 and 4.0 mg/kg rates are extremely high (two or three times that of baseline) with no evidence of response cessation.

Figure 4 presents cumulative records illustrating performance across the four pressure levels within a single session for placebo and each of the four dose levels. Reading down the chart, it is clear that performance in the first sequence

(1 ATA) again shows progressively increasing response rates as a function of dose. At 3 ATA performance remains unchanged for the placebo and 0.5-mg doses, but at the 1.0, 2.0, and 4.0 mg/kg dose levels severe disruptions are evident. At 5 and 7 ATA behavior is much improved over 1- and 3-ATA performance. In fact, performance at 5 and 7 ATA closely resembles baseline behavior except at the 4.0 mg/kg level, where elevated response rates persist.

The temporal discrimination that develops under the contingencies of the DRL schedule (the reinforced inter-response times were those with the highest relative frequencies) was evident in all of the animal baseline records. The temporal discrimination was maintained across the dose-response curve at the greater pressures (5 and 7 ATA) with only minor shifts to the shorter intervals (see Fig. 5). At 1- and 3-ATA pressure levels, however, the temporal discrimination was distorted in the presence of 1.0, 2.0 or 4.0 mg of the drug. Some discrimination remained intact, but large shifts toward the shorter intervals, with enormous increases in bursts of responses, were observed.

DISCUSSION

The present experiment has demonstrated that THC produces significant behavioral changes at 1-ATA pressure,
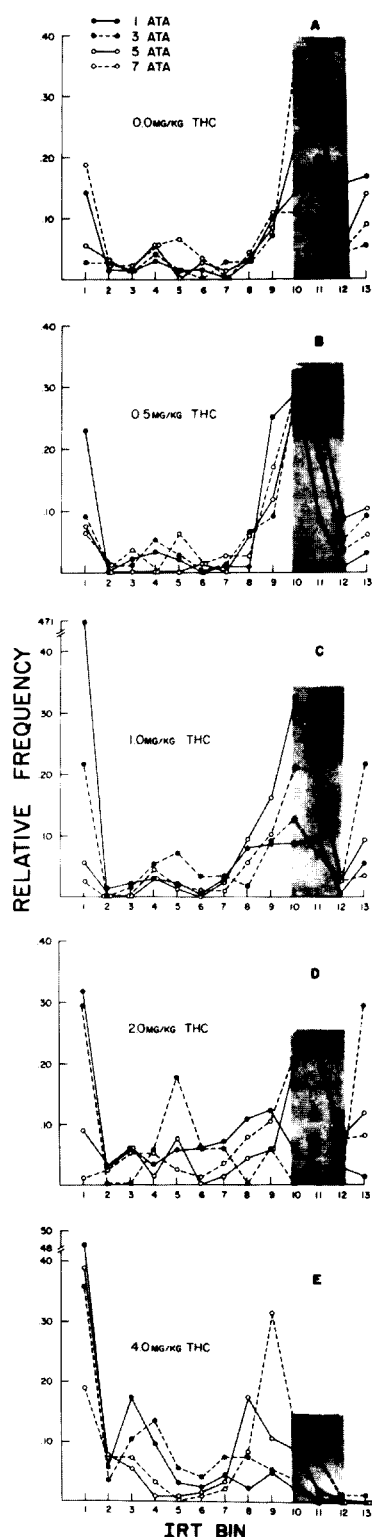
FIG. 5. Interresponse time distributions for one animal plotted for each of four pressures for placebo and four doses of Δ^9-THC. Shaded areas indicate reinforced bins.

but that when atmospheric pressure is increased the drug effects are reduced. Specifically, the increased response rates and decrements in accuracy of the timing response induced by the drug were reversed by the increased pressures of air.

Performance remained relatively constant across all pressures under the placebo condition in our study, yet previously reported findings [20] have shown these pressures to produce significant behavioral changes. The present experiment differed from the others because the greater pressures were successively approximated and the ultimate pressure change of 7 ATA was reached slowly, in 2-ATA increments. Apparently the 20-min stops at intermediate pressures (3 and 5 ATA) allow a gradual adaptation to occur so that the behavioral effects of N_2 are not evident.

The rationale for the reversal of THC effects is not completely clear. Cannabis compounds have been thought to have both excitant and depressant actions [6, 7, 12]. The increase in response rates and decrease in accuracy of responding on the DRL schedule observed here at 1 ATA supports previously reported findings under normal atmospheric conditions [13]. The implication is that THC distorts time perception, producing an overestimation of the passage of time and therefore premature responding, which is a phenomenon that has also been reported for human subjects [4].

In a series of studies evaluating the interaction of THC with stimulant and depressant classes of drugs [7], the investigators concluded that THC had sedative or tranquilizing actions. This conclusion was based on their findings that THC enhanced the CNS-depressant effects of pentobarbital and barbital and antagonized the toxic effects of methamphetamine stimulation. Earlier work at this laboratory [18] reported that stimulants (e.g., MAO-inhibitors, amphetamines) interact synergistically with high partial pressures of nitrogen when air is breathed at increased pressure and that depressants (e.g., alcohol and barbiturates) are antagonized by the same conditions. If we assume that THC is principally a depressant [7] and that hyperbaric air produces a stimulating effect [18], as has been suggested, then the reversal of the behavioral effects of the THC under hyperbaric conditions would be predictable.

The development of tolerance to the effects of marijuana on learned behavior maintained by food-reinforced schedules has been established [5,9]. Tolerance is usually developed by daily dosing. It has been reported [10] that daily doses can be gradually increased to levels that are lethal to naive animals but not disruptive to the behavior of tolerant animals. It is generally assumed that some residual concentration of the drug within an organism is necessary to maintain physiological tolerance; this is presumably the case in studies where daily doses are given. There have also been some reports of tolerance developing with weekly administrations in the pigeon [2] and the dog [11]. In the present study, however, the minimum interval between drug administrations was 7 days, and, since the drug effects were always evident at the 1-ATA conditions, physiological tolerance could not account for the observed effects. It is common in behavioral pharmacology, however, for the effects of a drug to vary from one determination to the next, even with lengthy intervals between administrations. Behavioral tolerance to the novelty of a drug may occur where behavior will tend to change to compensate for the effects of the drug. It is unlikely, however, that the observed antagonism of THC at increased pressure could be

attributed to behavioral tolerance since the time frame of the control (1 ATA) evaluation closely paralleled that of the pressure exposure and no improvements toward the end of the sessions were observed. Additional data indicate that baseline behavior does not begin to emerge until after a 4- to 5-hr period posttreatment.

Probably the best explanation of these results is that the drug effects were reversed by pressure. Whether the important variable is pressure itself or high partial pressures of nitrogen or oxygen is not yet clear. Presently, we are substituting helium for nitrogen and manipulating oxygen levels (which may change rate of THC metabolism) to gain insight into this phenomenon.

The results here may be similar to the reports of pressure reversal of anesthesia and analgesics at much greater pressures [8]. Perhaps the more sensitive behavioral baseline used here is capable of detecting such subtle changes at lower levels of pressure.

The diversity in the effects of THC make it a unique pharmacological compound to study in the hyperbaric environment. The determination of its interactions with increased pressure and partial pressure of gases may prove useful in the clarification of some hyperbaric phenomena.

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