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Relative Potency of Tetrahydrocannabinol Derivatives on Tonic Immobility in Chickens[1]

JACK D. MASER

Clinical Research Branch, National Institute of Mental Health, Rockville, MD 20852

GORDON G. GALLUP, JR.

Department of Psychology, State University of New York at Albany

WILLIAM R. THORN

Department of Chemistry, Tulane University, New Orleans LA 70118

AND

PATRICIA H. EDSON

Department of Psychology, University of Arkansas at Monticello, Monticello, AR 71655

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MASER, J. D., G. G. GALLUP, JR., W. R. THORN AND P. H. EDSON. *Relative potency of tetrahydrocannabinol derivatives on tonic immobility in chickens.* PHARMAC. BIOCHEM. BEHAV. 3(6) 1069–1072, 1975. – Chickens were given varying dosages of Δ^3-, Δ^8-, and Δ^9-THC and tested for duration of tonic immobility (TI). Although all derivatives had a profound facilitation effect, Δ^9-THC was the most potent. These results are unusual in that TI is a well documented fear-potentiated reaction and THC generally has tranquilization-like effects.

THC Derivatives Δ^3-THC Δ^8-THC Δ^9-THC Tonic immobility Catalepsy
Animal hypnosis Fear

IN recent years there has been a surge of interest in the neural and behavioral pharmacology of cannabinoids. Although modifications in behavior produced by tetrahydrocannabinol (THC) derivatives are now frequently, and in some cases consistently, reported, it is also relatively easy to locate contradictory evidence and failures to replicate earlier investigations. This is especially true for such standard behavioral tasks as maze learning [3, 23], acquisition of shuttlebox avoidance [12, 11], and schedule-controlled behavior [2, 6, 7, 29]. Miller and Drew [5, 21] should be consulted for recent reviews of this growing literature.

The present report concerns the rather potent effects of THC derivatives on the tonic immobility response (TI) in chickens. The essential features of the immobility response are consistent for every species studied (from insect through crustacea, avians, reptiles, lower mammals and primates). When manually restrained the subject passes from a struggling, active phase to one in which its posture appears frozen, and this immobility and lack of responsiveness may persist for a considerable period (about 10 min, on the average, in the chicken), despite removal of all restraint within 15 seconds after initial induction.

Tonic immobility, historically misconstrued as animal hypnosis, is an unlearned response whose complexity and relationship to other immobility behaviors has not often been appreciated [15]. Recent critical reviews suggest that brainstem mechanisms control the onset of the response [14], that its origin may be that of an evolved predator defense [25], and that variables generally recognized as producing enhancement or depression of fear will potentiate or reduce the duration of tonic immobility [8]. Among the many procedures having aversive properties which prolong the response are presence of a predator, loud noise, electric shock, suspension over a visual cliff, and presentation of conditioned fear stimuli. Taming, tranquilizers, and conditioned safety signals are among the procedures which decrease the magnitude of the reaction (see Gallup, [8] for a detailed review and other fear-related experiments).

Jaffe and Baum [13] found that hashish resin prolonged extinction of an avoidance response and suggested that fear may be enhanced by cannabinoids. This conclusion was based on faster swimming speed in an aversive situation, increased bolus count in a balance-beam test, and, at some dosages, reduced activity in an open field. Other investi-

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gators have concluded that Δ^9-THC may produce hyperemotionality [24]. It would be of interest, therefore, to determine the effect of THC on an innate behavior such as TI which is known to be potentiated by fear.

Another characteristic of most tonically immobile animals is waxy-flexibility and a trance-like or hypnotic gaze. This has given rise, primarily in the German literature of the 1900's, to the notion of animal catalepsy. More recently TI has been suggested as a model of human catalepsy [9, 10, 14, 19]. Cataleptic patients in remission often report that they had experienced considerable fear. Adverse reactions to marijuana in humans are rare but not unknown [30] and in some strains of the human species catalepsy may be induced [1]. These facts suggest that a catalepsy model might be further tested by observing TI following THC administration.

METHOD

Animals

The animals were 85 straight-run (unsexed) Production Red chickens received from a nearby hatchery at one day of age and housed in the laboratory in commercial brooders under a 14-hr light cycle. During rearing Purina chick chow (Growena) and water were available continuously. Testing occurred when the birds were 3–4 weeks of age.

Drug Synthesis and Injection Procedure

The (±)- Δ^3- α-methylhexyl and (−)-Δ^8 derivatives of tetrahydrocannabinol (Δ^3- and Δ^8-THC) were synthesized by the methods described in Thorn [28]. The (−)- Δ^9-THC isomer was provided by the Biomedical Research Branch, National Institute on Drug Abuse, Department of Health, Education, and Welfare. The latter compound was separated from its alcohol solvent and redissolved in propylene glycol, the standard vehicle for all THC compounds used here.

On the test day each bird was injected intraperitoneally with either 0.3 cc propylene glycol or a specified dose of either 0.3 cc Δ^3-, Δ^8-, or Δ^9-THC dissolved in propylene glycol. Chicks were not weighed prior to injection because handling affects tonic immobility [8]. Rather, each animal within a group received a constant 0.3 cc injection of a given compound and the dosage per kg was later determined as a mean for the group. There were 2 concentrations of Δ^3-THC: 1.50 mg and 0.75 mg. Three concentrations of Δ^8-THC were administered: 3 mg, 1.57 mg, and 0.75 mg. The Δ^9-THC was also given at 3 concentrations: 0.75 mg, 0.19 mg, and 0.095 mg.

All birds were weighed immediately following termination of the immobility response. Mean weight was then divided into the mg dosage administered to the group times 1000. The average mg/kg dose for each of the above groups was calculated to be as follows: for Δ^3-THC: 10.72 and 4.35; for Δ^8-THC: 20.34, 10.58, and 5.01; and for Δ^9-THC: 5.70, 1.34, and 0.63. The number of animals in each of the 9 groups to which the birds were randomly assigned varied from 8 to 10. The propylene glycol control group had 9 animals, the 20.34 mg/kg group of Δ^8- and the 1.34 mg/kg group of Δ^9-THC contained 8 animals, while all other groups were composed of 10 birds each. It is important to note that each bird received one injection and one test. Experimenters were unaware of which derivative, dose level and, in some cases, drug their animal had received.

Behavioral Testing

Following injection the bird was placed in an individual holding chamber, carried to a sound-attenuated test room and left alone for 25 min. At the end of this period the bird was removed from the chamber and placed on a table, where it was gently grasped and restrained on its right lateral surface. After 15 sec the experimenter slowly withdrew his hands and activated a stopwatch. If the initial induction was unsuccessful, the procedure was repeated up to 5 successive times. A bird that failed to become immobile was given a score of zero sec duration, weighed, and returned to a brooder. An upper limit of 2 hr was imposed on the duration of immobility.

RESULTS

Although each derivative of THC affected TI duration, there were clear differences in relative potency. Delta 9-THC, at dose levels comparable to Δ^3 produced the longest mean duration of immobility. Table 1 shows the mean durations for all dose levels. The mean duration for Δ^9-THC exceed those for comparable doses (0.75 mg) of Δ^8 and Δ^3 by 3788.8 and 1360.2 sec respectively. Following a square root transformation of the duration scores, separate analyses of variance were performed on data for each derivative and the propylene glycol control. After each F-value was obtained, Duncan's post-hoc test for unequal frequencies was applied to the means.

Neither dose of the Δ^3-THC was significantly different, but both exceeded the vehicle control group, $F(2,26) = 6.92$, $p<0.005$. The Δ^8-THC grouping was found to contain significant differences, $F(3,33) = 7.41$, $p<0.001$), the 2 highest dose groups being significantly different from the 6.01 mg/kg and vehicle control groups, but not from each other. The lowest dose of Δ^8-THC was not significantly different from the propylene glycol group. Analysis of variance also revealed significant differences among the 4 groups comprising the Δ^9-THC groupings, $F(3,31) = 4.95$, $p<0.01$. Post hoc testing revealed the 5.70 and 1.34 mg/kg levels significantly different from the 0.63 mg/kg and vehicle control groups.

An analysis of variance was also performed on the TI durations obtained at the 0.75 mg dose of each derivative, $F(3,33) = 7.83$, $p<0.001$. Post hoc testing revealed Δ^3- and Δ^9- to be significantly different from Δ^8-THC and the control group, but not from each other. Delta 8-THC, as previously described, was significantly different from the vehicle group.

DISCUSSION

The results demonstrate that all 3 THC derivatives affect TI and that the decreasing order of potency is Δ^9-, Δ^8-, and Δ^3-THC. The data do not lend themselves to an easy theoretical discussion but at least three speculative implications may be warranted. First, under certain conditions, as Jaffe and Baum [13] suggested, THC may potentiate rather than reduce fear. That humans do not generally find THC unpleasant seriously questions the generality of this implication.

Secondly, since THC facilitates TI, and is reported to induce cataleptic-like states in some humans (most notably Orientals) [1], the analogy between certain human psychopathological states and TI [9, 10, 14, 19] may hold promise. The fear and catalepsy explanations may not be

TABLE 1

TONIC IMMOBILITY DURATION MEANS AND STANDARD DEVIATIONS (IN SEC) FOR Δ^3-, Δ^8-, Δ^9-THC AND VEHICLE GROUPS. DOSAGE LEVELS CALCULATED AS AVERAGE MG/KG FOR EACH GROUP.

	Derivative								
	Δ^3-THC		Δ^8-THC			Δ^9-THC			Propylene Glycol
Mg/kg	10.72	4.35	20.34	10.58	6.01	5.70	1.34	0.63	0.5 cc
$\bar{X}$	4896.0	4461.3	5506.4	4763.1	2032.7	5821.5	3314.9	2899.4	1079.0
σ	2954.2	2689.5	2702.2	2040.9	1976.7	2333.1	2797.2	2826.4	760.7

mutually exclusive since many catatonic/cataleptic patients report high levels of fear while in the clinical state [10].

Finally, based on similarities between behavioral effects of hippocampectomy and anticholinergic compounds, Drew and Miller [5] hypothesized that the mode of action of THC is anticholinergic. Although no neurochemical measures were taken, the fear component of TI is thought to have a cholinergic base [20, 27]. It is therefore of interest that the induction of TI is itself known to be aversive [22] and that cholinergic systems are suspected to participate in behaviors associated with aversive contingencies [4, 17, 18].

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