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Time Course of Δ^9-Tetrahydrocannabinol Inhibition of Predatory Aggression[1]

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KILBEY, M. M., K. M. JOHNSON AND D. M. MCLENDON. *Time course of Δ^9-tetrahydrocannabinol inhibition of predatory aggression.* PHARMAC. BIOCHEM. BEHAV. 7(2) 117–120, 1977. – Three studies assessed the time course of inhibition of predatory aggression and changes in levels of brain serotonin following administration of Δ^9-THC. In Study One, six groups of six rats each were administered 1.25 mg/kg Δ^9-THC IV and frog-killing behavior was measured at six postinjection intervals: 30, 60, 90, 150, 210, and 270 minutes. In Study Two, four groups of six rats each were tested. Group One received a vehicle control injection and was tested immediately, i.e. zero-minutes, postinjection. The remaining groups received 1.25 mg/kg Δ^9-THC, and behavior was measured at 0, 15, and 30 min postinjection. In Study Three, two groups of six rats were treated with the vehicle or 1.25 mg/kg Δ^9-THC and sacrificed one minute postinjection. Additional drug groups were sacrificed at 30 and 210 min postinjection. Levels of 5-HT were determined in four brain sections: cortex, midbrain, medulla, and cerebellum. Significant inhibition of predatory aggression was found for groups tested at 0, 15, and 30 min postinjection. Brain levels of 5-HT in the midbrain and/or medulla were significantly increased over the same period.

Δ^9-Tetrahydrocannabinol Predatory agression Duration of effect Serotonin Midbrain Medulla

RECENT REVIEWS of the involvement of central neurotransmitters in aggression have suggested that serotonin (5-HT) is preponderantly inhibitory to several forms of aggressive behavior [5,17]. Several lines of evidence support this hypothesis. Administration of 5-hydroxytryptophan, the immediate precursor of 5-HT, blocked muricide in the rat [15]. Administration of d-l-parchlorophenylalanine, an inhibitor of tryptophan hydroxylase, facilitates shock-induced spontaneous fighting and predatory aggression in rats [1, 19, 20, 22]. Finally, compared with nonkillers, the turnover of 5-HT has been found to be slower in killer rats [24].

The effects of Δ^9-THC on endogenous brain 5-HT have been evaluated by several investigators. These investigators have reported that relatively high doses of Δ^9-THC elevated endogenous brain 5-HT [9, 21, 25], while lower doses have been observed to increase [9,12], decrease [8], or to have no effect on brain 5-HT levels [11,27]. In addition, several investigators have reported increases in whole brain 5-HT as a function of time [4, 9, 21] and one study reported that Δ^9-THC produced increases in 5-HT in the midbrain and cerebellum but was without effect in either the cortex or medulla plus pons [21]. To date the time course of the effects of Δ^9-THC on 5-HT levels in different brain areas has not been reported.

Additional pharmacological evidence has been provided by several studies utilizing Δ^9-THC. A dose-dependent inhibition of intraspecific attack behavior in mice in the absence of effects on spontaneous motor activity has been reported [11]. In rats Δ^9-THC reduced predatory aggression towards turtles [16] and mice and inhibited isolation-induced aggression in mice [2]. Similarly, we have previously reported that Δ^9-THC, at doses which did not impair rotorod performance, produced a dose-dependent inhibition of frog killing behavior in the rat [12]. This effect was associated with an increase in whole brain 5-HT levels [12].

In summary, the data suggest that Δ^9-THC inhibition of predatory aggression is associated with an increase in levels of brain 5-HT. The purpose of the study reported here was to confirm our previous findings of the correlation between Δ^9-THC-induced inhibition of frog killing behavior and elevation of brain 5-HT levels [12] and to extend these observations to determine if the time duration of this behavioral effect of Δ^9-THC was correlated with an alteration in the regional brain concentration of 5-HT.

METHOD

In Study One, 36 male Long-Evans rats, approximately 250 g weight, were maintained on 23 hr food deprivation with ad lib access to water until their weight had stabilized at 80% original body weight. The animals were singly caged and exposed to a 12-hr light-dark cycle. Five six-min training trials, separated by a one-min intertrial interval, were completed daily during the late afternoon. A two-to-three inch frog, *Rana pipins* (northern), was dropped into the home cage, and attack and kill latencies were recorded. Kill latency was defined as the amount of time necessary for the rat to immobilize the frog plus 20 sec during which the rat repeatedly bit the frog without an apparent response

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from the frog. The frog was then removed, and if it were still breathing it was immediately killed by the experimenter. If, upon removal, the frog was mobile the kill latency was recorded as 360 sec. Kill latencies were compared on a day-to-day basis using the Sandler's A statistic [18]. After three days in which latencies were stable (e.g., Sandler's A $p>0.025$, 2 tailed) testing was initiated. All animals received a tail vein injection of 1.25 mg/kg THC suspended in PVP, a 3% polyvinylpyrrolidone K-30 in saline vehicle [3], at a volume of 1 cc/kg. Attack and kill latencies were measured for five trials at the following postinjection intervals: 30, 60, 90, 150, 210 and 270 min, with six animals assigned randomly to each group.

In Study Two, twenty-four male Long-Evans rats were subjected to a paradigm identical to that of Study One. Six animals were assigned randomly to 1.25 mg/kg THC groups for testing at 0, 15 and 30 min postinjection. A fourth group of six animals received PVP and were tested 0 min postinjection.

In Study Three, twenty-eight male Long-Evans rats were maintained under food and housing conditions identical to those in Studies One and Two. The animals, randomly assigned to groups of six animals each, were injected with 1.25 mg/kg THC and were sacrificed at the following postinjection times: 1, 30 and 210 min. An additional group of six rats was injected with PVP and sacrificed one min postinjection. The animals were decapitated, and the brains were sectioned [6] into four parts: cortex, midbrain, medulla, and cerebellum; and frozen for fluorimetric analysis of 5-HT [23,25].

RESULTS

As analysis of variance [26] for the mean kill latencies for the 30 min groups of Studies One and Two showed no significant differences ($p>0.05$) between the groups on either the pretest, $F(1,5) = 0.36$, or test day, $F(1,5) = 1.9$, the data for the studies were combined. To maintain a group size of six in the 30 min group, three animals were chosen randomly from each study.

Analysis of variance for the pretest mean kill latency as a function of group assignment showed that there were no differences among the groups prior to drug treatment, $F(8,45) = 1.33$, $p>0.05$. Analysis of variance for the test day data indicated a significant difference among the groups as a function of the postinjection interval at which the animals were tested, $F(8,45) = 43.01$, $p\leq 0.01$. For both tests, F max tests [34] indicated no violation of the homogeneity of variance assumption, F max (9,5) = 20.74 and 7.20, $p>0.05$.

Individual comparisons using the Newman-Keuls statistic [26] indicated that the mean kill latencies for the THC groups tested at 0, 15 and 30 were significantly longer ($p<0.05$) than all other groups. In addition, the mean kill latencies for the 0 and 15 min groups were significantly longer than those of the 30 min groups ($p\leq 0.05$). These data are presented in Table 1.

The concentration of 5-HT in the four brain areas was analyzed by comparing each postinjection THC group with the PVP group using the F test. These analyses showed that levels of 5-HT for the one min THC group were increased significantly for the midbrain and medulla areas, $F(1,10) = 7.25$ and 4.96, $p\leq 0.05$, respectively. A significant increase of 5-HT was found for the medulla area of the 30 min group as well, $F(1,10) = 5.58$, $p\leq 0.05$. These data are presented in Table 2.

TABLE 1

MEAN KILL LATENCY (SECONDS) FOR EIGHT DURATIONS (MIN) POSTINJECTION FOLLOWING ADMINISTRATION OF THC AND ONE DURATION FOLLOWING PVP ADMINISTRATION

Time	Drug	$\bar{X}$	SD
0	PVP	70.4	48.94
0*	THC	346.5	33.06
15*	THC	333.8	41.42
30*	THC	267.1	77.95
60	THC	89.6	52.39
90	THC	80.0	58.16
150	THC	49.2	35.68
210	THC	62.9	24.69
270	THC	65.0	21.66

*$p\leq 0.05$.

TABLE 2

MEAN NG/G 5-HT FOR FOUR BRAIN AREAS AT THREE DURATIONS (MIN) POSTINJECTION OF THC (1.25 MG/KG) OR PVP

Time	Drug	Cortex	Midbrain	Medulla	Cerebellum
1	PVP				
	$\bar{X}$	430.0	681.1	682.8	100.8
	SD	73.6	107.0	120.5	54.9
1	THC				
	$\bar{X}$	415.3	837.8*	890.6*	151.8
	SD	63.5	94.0	194.1	66.7
30	THC				
	$\bar{X}$	435.1	836.6	811.3*	98.0
	SD	104.6	150.7	56.4	42.6
210	THC				
	$\bar{X}$	382.3	734.5	759.5	113.8
	SD	110.3	72.6	128.5	60.9

$p\leq 0.05$.

DISCUSSION

The results of this study are in agreement with other work which has shown that Δ^9-THC attenuates predatory aggression in rats [1, 12, 16]. This study also verifies our earlier observation [14] that Δ^9-THC (1.25 mg/kg) elevates brain 5-HT and reduces frog killing in the rat. In addition, this study adds strength to the Δ^9-THC-induced correlation between inhibition of predatory aggression and increased brain 5-HT levels by extending this correlation to include the time duration of both effects. This study also identifies areas of the brain (midbrain, medulla, and pons) which contain serotonergic cell bodies as those responsible for increases in whole brain 5-HT concentrations. These areas contain structures which are thought to be central for the expression of aggressive behavior [5]. Thus, these results lend support to the hypothesis that 5-HT mechanisms inhibit predatory aggression [17].

Although the mechanism by which Δ^9-THC elevates brain 5-HT levels in this species remains to be elucidated, we have recently obtained data in the mouse which suggest that Δ^9-THC produces an increased uptake by the brain of plasma tryptophan [10]. Since tryptophan hydroxylase is normally unsaturated with precursor, this increase in brain tryptophan resulted in an increased formation of brain 5-HT without significantly altering the actual synthesis rate of 5-HT [10]. This effect of Δ^9-THC is consonant with the finding that administration of 5-hydroxytryptophan blocked muricide in rat [15].

In spite of the obvious correlations between the suppression of aggressive behavior and increases in brain serotonin reported here and elsewhere [12], it is very difficult to causally relate these effects of Δ^9-THC. It is entirely possible, even probably, that the effects of Δ^9-THC on predatory aggression are the result of alterations in the balance between several neurotransmitter systems. Effects of Δ^9-THC on norepinephrine, dopamine, and acetylcholine synthesis and metabolism have been described in the literature. (For a review of the neuropharmacology of cannabis, see reference [7].)

In view of the far-ranging neuropharmacological effects of Δ^9-THC, it could be argued that impairment of predatory aggression could be the result of diminished motor activity and/or coordination [14] or decreased preception of cues necessary to elicit aggressive behavior [16]. However, we have obtained results in the rat using doses of Δ^9-THC similar to those used in the present study which argue against the possibility that the effects of Δ^9-THC on predatory aggression are due to a nonspecific incapacitation of the rat's ability to carry out the aggressive behavior. For example, the activity of rats, as measured in a photocell apparatus, was not influenced by Δ^9-THC in doses ranging from 0.25 mg/kg to 2.00 mg/kg (IV) [12]. Similarly, rotorod performance by female rats was unimpaired by doses of Δ^9-THC which produced increases in both attack and kill latencies [12]. In addition, we have observed that Δ^9-THC inhibited aggression at dose levels which did not impair a learned preference for the food rewarded end of a T-maze; a behavior which appears to be dependent upon the utilization of perceptual cues [13].

In summary, the data presented here confirm our previously reported Δ^9-THC-induced dose-dependent correlation between increased levels of brain serotonin and attenuated levels of predatory aggression and extend this correlation to include time duration of these effects. We also present data which suggest that brain areas rich in serotonergic cell bodies are sensitive to Δ^9-THC and are probably responsible for the increases in whole brain 5-HT observed by our laboratory in a previous study.

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