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Footnotes:

Abbreviations used: THC, delta-9-tetrahydrocannabinol;
Tween-80, polysorbate 80;
CTAB, cetyltrimethyl ammonium bromide.

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Recent behavioral and physiological studies with delta-9-tetrahydrocannabinol (THC) are extensive and several suggest short term memory impairment (TINKLEBERG, MEIGES, HOLLISTER, and GILLESPIE, 1970; MEIGES, TINKLEBERG, HOLLISTER, and GILLESPIE, 1970; WEIL and ZINBERG, 1969; SCHECKEL, BOFF, DAHLEN and SMART, 1968; ZIMMERBERG, GLICK, and JARVIK, 1971). Studies of metabolic changes in brain following THC administration are few in number (HOLTZMAN, LOVELL, JAFFE, and FREEDMAN, 1969) with the most recent studies suggesting inhibition of macromolecular synthesis (LUTHRA, ROSENKRANTZ, MUHILLY, THOMPSON, and BRAUDE, 1971; JAKUBOVIČ and McGEER, 1972a; JAKUBOVIČ and McGEER, 1972b). Inhibitors of RNA and protein synthesis have been implicated in the impairment of long-term memory (BARONDES and COHEN, 1968; FLOOD, ROSENZWEIGH, BENNETT, and ORME, 1972; HORN, ROSE, and BATESON, 1973) and have been used widely in memory research.

We have studied ^{14}C-L-valine and ^{3}H-5-uridine incorporation into rat brain following acute (intracranial and smoke inhalation) and chronic (intraperitoneal) administration of THC. Our findings suggest other experiments that may be done to elucidate the biochemical effects of THC in rat brain.

Materials and Methods

Male adult rats (Sprague-Dawley) each weighing about 200 grams were obtained from Charles Rivers Co., San Mateo, California. ^{3}H-5-uridine (1.0 mC/ml, 105.3 mC/mg) and U-^{14}C-L-valine (0.1 mC/ml, 0.45 mC/mg) were obtained from New England Nuclear. Delta-9-tetrahydrocannabinol in a 200 mg/ml alcohol solution was supplied by the National Institute of Mental Health, Washington, D.C. Gas liquid chromatography taken every four weeks from date of receipt of THC showed a single peak matching the original NIMH supplied analysis, indicating stability of THC under storage conditions.

Sesame seed oil (Hollywood Health Food) and Tween-80 solution (Mann Research Laboratories) and sterile saline (0.9% NaCl, Cutter Laboratory) were used in making an emulsion for intraperitoneal route. THC and ^{14}C-L-valine were stored at -21°C, while ^{3}H-5-uridine was kept in ethyl alcohol at +2°C (KUPOLDOVÁ and DĚDKOVÁ, 1971) and diluted to 0.25 mC/ml before use.

THC was injected intracranially at a dosage of 10 µl per rat (10 mg/kg body weight). Control rats received equal volumes of absolute alcohol. Animals were sacrificed after 1, 2, 3, 4, and 5 hours. Thirty minutes before decapitation 50 µl of ^{14}C-L-valine was injected subcutaneously. Brains were excised and immediately frozen on dry ice to halt enzyme activity before transfer to a freezer at -21°C.

In another set of acute experiments, cigarettes containing 50 µl (10 mg) of THC in ethanol were prepared. Control cigarettes contained an equal volume of absolute alcohol. Animals were placed in a sealed chamber. Smoke was drawn into the cham-

ber using a flow rate of 155 cc air/min for ten seconds of every minute for 12 min. The use of a double stopcock enabled air to be drawn in at the same rate for the remaining 50 sec of every minute. Animals were sacrificed at 1, 2 and 3 hr after inhalation. Otherwise, procedures were the same as described above.

In a final set of experiments, the effects of chronic administration of THC was determined. Rats were injected intraperitoneally with an emulsion consisting of 5 ml of 200 mg/ml THC in ethanol solution, 50 ml of sesame seed oil,, 5 ml of Tween-80 solution, and 90 ml of sterile saline (0.9% NaCl solution) (ROSENKRANTZ, THOMPSON, and BRAUDE, 1972). This was emulsified by sonification (Heat Systems-Ultrasonics, Inc. sonifier cell disruptor W185) for about 1 min, after which there was a brief wait followed by an additional 30 sec of sonification. This formed a milky-type emulsion, which was bottled 6 to 8 ml per ampule and then stored in a refrigerator. New supplies were made every 10 to 15 days as needed. Rats received a daily dose of 10 mg/kg. (Animals were weighed daily during the first 10 days and thereafter every other day.) Animals were sacrified at 10, 20, and 30 days. Thirty min before decapitation, half of the animals received subcutaneously 50 µl of ^{14}C-L-valine. The other half of the animals received 10 µl of ^{3}H-5-uridine intracranially (5 ul bilaterally). All brains were excised and stored as described above.

The TCA protein extraction procedure used was described by FLOOD, ROSENZWEIG , BENNETT, and ORME (1972). Incorporation of ^{14}C-L-valine into protein was calculated as the percent of

$$\left(\frac{\text{TCA precipitate dpm}}{\text{TCA precipitate dpm + supernatant dpm}}\right) \times 100$$

.(FLEXNER,

FLEXNER, ROBERTS, and de la HABA, 1964).

For RNA, brains were homogenized to a concentration of 25 mg/ml in 0.15M EDTA with pH adjusted to 6.0 by addition of NaOH pellets. Homogenate aliquots (500 µl) at 0°C were precipitated with an equal volume of cetyltrimethyl ammonium bromide (CTAB) at room temperature. (Both EDTA·Na_2 and CTAB were supplied by Matheson.) The precipitate was kept at 0°C for at least 30 min and then filtered through a Millipore apparatus (Whitman GFK filters 2.4 cm) with a water wash and at least 6 ml of ethanol saturated with potassium acetate removing free trinucleotides and forming potassium salts of nucleic acids. Filters were then placed in counting vials, hydrolyzed in 0.3N KOH at 37°C for one hour, and then neutralized with 100 µl of 3.0N HCl before adding 13 ml dioxane scintillation fluid (250 ml EtOH, 400 ml 1,4 dioxane, 50 g naphthalene, 50 ml permafluor from Packard, and diluted to one liter with toluene). Cabosil Thixotropic silica gel (New England Nuclear) was added which suspended filters in solution. We found this to be necessary for accurate radioactivity estimates. Aliquots (150 µl) of the homogenate were placed in counting vials and dried under a heat lamp to remove the ^3H_2O formed from either the self-decomposition or metabolism of ^{3}H-5-uridine. The homogenate was also hydrolyzed and neutralized as above before 13 ml of the dioxane scintillation fluid was added. Samples were counted in a Packard Tri-Carb Liquid Scintillation Spectrometer Model 3375. Incorporation values were calculated as $\left(\frac{\text{precipitate dpm}}{\text{homogenate dpm}}\right) \times 100$.

Results

Since THC does not pass easily through the blood-brain barrier, several modes of administration were employed. Chronic and acute studies were done for comparison to previous *in vivo* and *in vitro* studies.

Incorporation of ^{14}C-L-valine appears to be unchanged following, 1) intracranial injection of -THC into the brains of approx. 200 g rats (Table 1), 2) inhalation of 1.7 mg of THC burned in a smoking chamber (Table 1), and 3) daily intraperitoneal injection of 10 mg THC/kg body weight for up to 30 days (Table 2). The latter experiment also showed no effect on a group of rats injected in the same manner when the incorporation of ^{3}H-5-uridine was measured (Table 2). Preliminary experiments using intracranial injection also indicate no effect on ^{3}H-5-uridine incorporation. Inhalation studies have not been done to measure RNA synthesis.

Insert Tables 1 & 2 here-->

Discussion

While the behavioral response of the experimental rats was not uniform, generally they exhibited ataxia, hypersensitivity to tactile and auditory stimuli, and diminished motor activity. These observations are consistent with behavioral studies (TINKLEBERG, MELGES, HOLLISTER, and GILLESPIE, 1970; MELGES, TINKLEBERG, HOLLISTER, and GILLESPIE, 1970; WEIL and ZINBERG, 1969; SCHECKEL, BOFF, DAHLEN, and SMART, 1968; ZIMMERBERG, GLICK, and JARVIK, 1971).

Although three out of the fourteen comparisons (the various times of animal sacrifice) showed a statistically significant treatment effect ($p < .01$, t-test), we feel that due to the erratic nature of the results and the small number of rats emphasis should not be placed on this result. It should also be noted that an average of all THC rats vs. control rats showed no significant difference in each of the four sets of experiments.

Some previous studies have indicated significant inhibition in RNA and protein synthesis and decreased whole brain content of RNA and protein. LUTHRA, et al., (1971), used daily doses of 50-500 mg/kg administered by oral gavage for 28 and 91 days. A dose related decrease of about 30% was found for protein and RNA in the brain. They suggested that THC might stimulate the breakdown and/or prevention of syntheses of these macromolecules. Very recently, LUTHRA, et al., (1973) have reported that chronic administration of delta-9-THC caused a 9-14% increase in RNA in cerebellum of rats.

JAKUBOVIČ and McGEER (1972a) doing in vitro studies, incubated slices of rat brain tissue with 0.1 mM THC and ^{14}C-L-leucine. They reported a significantly lower leucine incorporation into protein for THC animals than for controls. Similar values were obtained for the incorporat on of ^{3}H-5-uridine into nucleic acids in the presence of THC. Using infant rats they reported a significant inhibition in in vivo studies of the uptake of uridine and leucine into RNA and protein, respectively, 3 to 4 hours after intracranial injection of 20 mg THC/kg. CARLINI and CARLINI (1965) found no effect on RNA concentration in the brain either when 10 or 100 mg/kg of crude marijuana extract was injected intraperitoneally, but did report a large decrease in DNA concentration.

Our studies do not support the hypothesis that the THC affects brain metabolism by inhibiting RNA and protein synthesis. We did not find significant inhibition of either RNA or protein synthesis in whole brain tissue

following acute or chronic experiments, employing three modes of administration. MORIMOTO and BENNETT (1973, personal communication) have found little or no effect of THC on DNA, RNA, or AChE activity in rat brain after daily intraperitoneal administration of 10 mg/kg for 30 days.

The similarity of THC to 5-hydroxytryptamine suggests that neurotransmitters may be affected rather than the synthesis of RNA and protein (LUTHRA, et al., 1971). We are extending our experiments to determine the concentration of THC in rat brain after intraperitoneal administration.

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TABLE 1

EFFECT OF A SINGLE EXPOSURE TO THC ON THE INCORPORATION OF VALINE INTO RAT BRAIN

	Hours after the Exposure					Average of all times tested
	1	2	3	4	5	
BRAIN INJECTION						
Experimental	51 ±2	50 ±2	51 ±3	54 ±3	54 ±1	52 ±3
	N=5	N=4	N=9	N=6	N=4	N=28
Control	58 ±3	47 ±3	57 ±5	50 ±3	49 ±1	53 ±6
	N=6	N=5	N=8	N=4	N=3	N=26
SMOKE INHALATION						
Experimental	58 ±2	49 ±3	52 ±4			53 ±5
	N=4	N=5	N=5			N=14
Control	53 ±6	56 ±3	53 ±3			54 ±4
	N=5	N=3	N=5			N=13

TABLE 2

EFFECT OF CHRONIC INTRAPERITONEAL INJECTION OF THC ON THE RATE OF VALINE AND URIDINE UPTAKE IN RAT BRAIN

	Consecutive Days of Injection		
	10	20	30
% INCORPORATION OF VALINE			
Experimental	51 ±3	49 ±4	47 ±3
	N=4	N=4	N=5
Control	46 ±7	49 ±3	48 ±4
	N=4	N=4	N=5
%INCORPORATION OF URIDINE			
Experimental	33 ±3	20 ±4	34 ±5
	N=4	N=4	N=4
Control	33 ±7	22 ±8	31 ±5
	N=4	N=3	N=5

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