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Δ^9-Tetrahydrocannabinol-Induced Changes in Brain Ribosomes

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Δ^9-Tetrahydrocannabinol-Induced Changes in Brain Ribosomes. PODDAR, M. K., MITRA, G., AND GHOSH, J. J. (1978). *Toxicol. Appl. Pharmacol.* **46**, 737–757. The action of Δ^9-tetrahydrocannabinol (Δ^9-THC) on the stability of rat brain cortex and hypothalamic ribosomes has been studied. Δ^9-THC, both under *in vivo* and *in vitro* conditions, does not significantly affect the chemical composition and uv absorption characteristics of the brain cortex and hypothalamus. Studies with Δ^9-THC reveal that: (a) at low doses (2 mg/kg under *in vivo* conditions; 0.2 and 0.5 mg/g of tissue slices under *in vitro* conditions) the ribosomal particles are less susceptible to breakdown and the ribosomal RNAs have a greater proportion of hydrogen-bonded structure than those of the corresponding control tissues; (b) at high doses (10 and 50 mg/kg under *in vivo* conditions; 1.0 and 5.0 mg/g of tissue slices under *in vitro* conditions) and under chronic treatment with Δ^9-THC (10 mg/kg/day for 21 consecutive days) the ribosomal particles become more susceptible to breakdown with release of protein, RNA, and acid-soluble nucleotides than do the ribosomes of the corresponding controls, and they have a smaller proportion of hydrogen-bonded structure than the ribosomal RNAs of control brain cortex and hypothalamus. These differential effects of Δ^9-THC, depending on the concentration and the condition of treatment of the brain ribosomes, may be ascribed to the difference in the degree and mode of hydrophobic interaction between the Δ^9-THC molecule and the ribosomal components.

Δ^9-Tetrahydrocannabinol (Δ^9-THC), the well-known pharmacologically active component of *Cannabis*, due to its high lipophilicity, has been found to be accumulated at a measurable concentration in brain and other lipid-rich tissues (Agurell *et al.*, 1969; Klausner and Dingell, 1971). While studying the effect of Δ^9-THC on behavioral changes, McIssac *et al.* (1971) observed a dose–response relationship indicating that at lower doses Δ^9-THC produces an anxiolytic and euphoric action accompanied by a disruption of time perception; medium doses cause stimulation and high doses produce hallucinatory signs. It has also been reported in the last few years that Δ^9-THC changes brain nucleic acid (Carlini and Carlini, 1965; Jakubovic and McGeer, 1972) and alter memory and acquisition processes involved in the storage of information in humans and animals (Hollister, 1971; Kolansky and Moore, 1971; Melges *et al.*, 1970). Previous studies from our laboratory have shown that different convulsant and antidepressant drugs destabilize the ribosomal particles of brain cortex slices (Datta and Ghosh, 1963a,b,c, 1965, 1970; Ghosh *et al.*, 1965). Recent studies on the alteration by Δ^9-THC of protein–RNA structural units necessary for brain function (Yugal *et al.*, 1971) led the authors to investigate the biochemical mechanism of action of Δ^9-THC at the level of stability of the cytoplasmic ribosomes of brain cortex and hypothalamus. In the

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present investigation, both *in vivo* and *in vitro* actions of Δ^9-THC on the structural integrity of brain ribonucleoprotein particles, along with the hydrogen-bonded structure of rRNA of rat brain cortex and hypothalamus, have been studied.

METHODS

In vivo Δ^9-THC treatment. Male albino rats of the Charles Foster strain, weighing about 110–125 g, were injected ip with pure (99%) Δ^9-THC at doses of 2, 10, and 50 mg/kg for acute experiments. Dosages were prepared by suspending the pure Δ^9-THC at a concentration of 0.4, 2, and 10 mg/ml in normal saline containing 1% Tween 80. The suspensions were administered in a volume of 0.5 ml/100 g body wt. The animals were sacrificied by decapitation 6 hr after the injection. Rats of the chronic experimental group were treated with pure Δ^9-THC suspension at a dose of 10 mg/kg/day, for 21 consecutive days by the same route. The animals were decapitated 6 hr after the last injection. Animals of the control group corresponding to the acute and chronic groups received an injection of an equal volume of saline–Tween 80 vehicle and were decapitated under similar conditions. After decapitation the cortex and hypothalamic portions of the brain were separated and used to isolate the corresponding ribosomes.

In vitro Δ^9-THC treatment. The cortex and hypothalamic portions of the brain of freshly slaughtered adult rats (100–125 g body wt) were removed and cut into thin slices, 0.5 mm thick, in the cold (0–4°C). Approximately 10 g of wet weight of brain cortex or hypothalamic slices was suspended in 100 ml of Krebs–Ringer buffer, pH 7.0, containing 0.5 M glucose, which was previously saturated with 95% O_2 and 5% CO_2. Δ^9-THC at doses of 0.2, 0.5, 1.0, and 5.0 mg/g of wet tissue slices was added to the flasks containing the suspension of the respective tissue slices. The properties of control slices were checked by separate manometric experiments carried out under similar conditions. In the control experiment only saline–Tween 80 vehicle was added and the incubation was carried out under similar condition. After incubation at 37°C for 2 hr the slices of cortex or hypothalamus were collected by centrifugation and washed three to four times with 100-ml portions of Krebs–Ringer buffer. Unless otherwise stated, Δ^9-THC treated cortex or hypothalamus slices indicate glucose plus Δ^9-THC-treated slices, whereas untreated (normal) slices mean slices treated only with glucose plus vehicle (saline–Tween 80).

Preparation of ribosomes. Ribosomes were prepared from the untreated and Δ^9-THC treated brain cortex and hypothalamus by the method of Datta and Ghosh (1963b).

Extraction of ribosomal RNA. rRNAs of both brain cortex and hypothalamus were extracted by the method of Kirby (1956) as described by Littauer and Eisenberg (1959). The precipitated rRNA of each tissue was dissolved in water and further purified by passing through a column of Sephadex G-25 which was equilibrated previously with water as described by Martin (1966).

Chemical estimations. Protein was determined by the method of Lowry *et al.* (1951) using crystalline bovine serum albumin as standard. Nitrogen was estimated by the modified micro-Kjeldahl method described by Ma and Zuazaga (1942). Phosphorus was determined by the digestion of RNAs according to the method of King (1932) and inorganic phosphorus was estimated by the method of Lowry *et al.* (1954). RNA was estimated by the orcinol method of Mejbaum (1939) and also by the uv spectrophoto-

metric method of Hotchkiss (1957). Hexoses and hexosamines were determined by the methods of Hess and Slade (1956) and Elson and Morgan (1933), respectively. Nucleotides obtained after hydrolysis with 0.3 M KOH at 37°C for 18 hr were separated by paper chromatography by the method of Magasanik *et al.* (1950) and the amounts of the respective nucleotides were calculated using the factors given by Elson *et al.* (1954). The concentration of acid-soluble nucleotides was determined spectrophotometrically assuming an absorbance value of 33 at 260 nm for a solution containing a mixture of nucleotide after hydrolysis of 1 mg of yeast RNA/ml in 5 mM Mg-cacodylate buffer, pH 7.0. Amino acids were determined by the ninhydrin method of Moore and Stein (1948).

Enzymic assays. The ribonuclease (RNase) activity was measured by the method of McDonall (1955). The alkaline phosphomonoesterase (PMase) activity was assayed by the method of Bessey *et al.* (1946). The alkaline phosphodiesterase (PDase) activity was measured by the method of Frisch-Niggemeyer and Reddi (1957) with bis-*p*-nitrophenyl phosphate as the substrate. The procedure was basically the same as that for PMase, which has been described in detail by Datta and Ghosh (1963c) and Datta *et al.* (1969).

Alkaline hyperchromicity. Ribosomes and extracted RNAs of both brain cortex and hypothalamus were hydrolyzed with 0.3 M KOH solution for 18 hr at 37°C followed by removal of protein by treatment with ice-cold 2 N perchloric acid. After neutralization uv absorption was measured at 260 nm.

Measurement of thermal hyperchromicity. The extracted rRNAs (20–25 μg/ml) were dissolved in 28 mM acetate veronal buffer, pH 7.0 (Michaelis, 1931) and heated for 20 min in a thermostatic heating chamber attached to the Beckman DU spectrophotometer in stoppered 1-cm cuvettes. The absorbance at 260 nm was measured over the range of temperatures between 30 and 85°C during heating and also during cooling. The percentage increase of absorbance at 260 nm or thermal hyperchromicity value between 30 and 85°C was calculated according to the method of Doty *et al.* (1959).

Measurement of formaldehyde hyperchromicity. Formaldehyde hyperchromicity was measured according to the methods of Boedtker (1967) and Cox (1969). The extracted rRNAs (20–25 μg/ml) were dissolved in 28 mM acetate Veronal buffer, pH 7.0, containing 2% (w/v) formaldehyde and solutions were kept for 6 hr at 30°C. The corresponding control blanks were treated similarly. At definite time intervals, the absorbance at 270 nm was measured in a Beckman DU spectrophotometer in stoppered 1-cm cuvettes. The increase of absorbance of the solutions almost ceased at about 6 hr. Next, the solutions were heated in the stoppered cuvettes in a thermostatic chamber maintained at 70°C for 4 hr. The absorbance of solutions was measured at 270 nm at definite time intervals during heating as well as during cooling. The percentage increase absorbance at 270 nm or formaldehyde hyperchromicity between 30 and 70°C was calculated.

RESULTS

The uv absorption spectra and the chemical composition of the ribosomal particles and total rRNA preparations of Δ^9-THC-treated brain cortex and hypothalamus as estimated from the contents of nitrogen, phosphorous, protein, RNA, hexoses, hexosamines, and acid-soluble nucleotides such as adenylate, cytidylate, guanylate, and uridylate showed no significant difference from those of the control tissues under both *in vivo* and *in vitro* conditions of treatment (Data not shown).

TABLE 1

In Vivo AND *In Vitro* EFFECT OF Δ^9-THC ON SPECIFIC ACTIVITIES OF RIBOSOMAL ENZYMES OF RAT BRAIN CORTEX AND HYPOTHALAMUS[a]

Treatment	Dose	Specific activities (%)					
		Brain cortex[b]			Hypothalamus[b]		
		RNase (pH 5.5)	PMase (pH 8.0)	PDase (pH 9.5)	RNase (pH 5.0)	PMase (pH 8.3)	PDase (pH 10.0)
In vivo							
Control (saline–Tween 80)	—	100	100	100	100	100	100
Δ^9-THC	2 (mg/kg)	140	148	128	125	130	120
	10 (mg/kg)	78	62	75	82	76	80
	50 (mg/kg)	64	55	63	72	60	70
	10 (mg/kg/day)[c]	56	48	52	65	54	61
In vitro							
Control (saline–Tween 80)	—	100	100	100	100	100	100
Δ^9-THC	0.2 (mg/gm) of tissue slices)	167	187	142	148	170	132
	0.5 (mg/kg of tissue slices)	157	168	128	130	140	121
	1.0 (mg/kg of tissue slices)	72	65	63	78	72	50
	5.0 (mg/kg of tissue slices)	57	48	42	67	58	52

[a] Results expressed as mean of six different experiments. The enzymic activities of ribosomes of brain cortex and hypothalamus were assayed at their respective optimum pH values. The assay system of: (a) RNase contained 0.5 mg of ribosomal protein, 2.5 mg of purified dialyzed yeast RNA, and 0.014 M acetate veronal buffer of respective pH in a total volume of 1 ml; (b) PMase contained 0.5 mg of ribosomal protein, 5 mM *p*-nitrophenyl phosphate, and 0.014 M acetate Veronal buffer of respective pH in a total volume of 2 ml; (c) PDase contained 0.5 mg of ribosome protein, 5 mM bis-*p*-nitrophenyl phosphate, and 0.014 M acetate Veronal buffer of respective pH in a total volume of 2 ml. Other details are described under Methods.

[b] In expressing the results, the ribosomal enzyme activities of control experiment are taken as 100%.

[c] Treatment was continued for 21 consecutive days.

It is evident from the results of Table 1 that Δ^9-THC, at low doses, under both [*in vivo* (2 mg/kg) and *in vitro* (0.2 and 0.5 mg/g of wet tissue slices] conditions of treatment, increased the specific activities of RNase, PMase, and PDase of both brain cortex and hypothalamic ribosomes. At higher doses, under both *in vivo* (10 and 50 mg/kg) and *in vitro* (1.0 and 5.0 mg/g of wet tissue slices) conditions of treatment and also during chronic treatment with Δ^9-THC, the specific activities of these three enzymes from brain cortex and hypothalamus are depressed. Table 1 also shows that stimulation or inhibition of ribosomal enzymes, depending on the concentration of Δ^9-THC, is found to be greater in brain cortex ribosomes than in the hypothalamic ribosomes.

TABLE 2

EFFECT OF Δ^9-THC ON SPECIFIC ACTIVITIES OF RIBOSOMAL ENZYMES OF RAT BRAIN CORTEX AND HYPOTHALAMUS[a]

	Specific activities (%)					
	Brain cortex			Hypothalamus		
Conditions of treatment	RNase (pH 5.5)	PMase (pH 8.0)	PDase (pH 5.0)	RNase (pH 5.0)	PMase (pH 8.3)	PDase (pH 10.0)
Control ribosomes (glucose, 0.5 M)	100	100	100	100	100	100
Treated ribosomes						
Glucose (0.5 M) + Δ^9-THC (5 μg/mg of ribosomal protein)	105	106	104	103	104	104
Glucose (0.5 M) + Δ^9-THC (10 μg/mg of ribosomal protein)	92	96	95	90	97	96
Glucose (0.5 M) + Δ^9-THC (50 μg/mg of ribosomal protein)	88	93	90	88	94	92

[a] Data represent the average of six determinations of different ribosomal preparations. The enzymic activities were assayed in the presence of Δ^9-THC at different concentrations (μg/mg of ribosomal protein) at their respective optimum pH values. The assay system and other details are described in Table 1.

Table 2 shows that when fresh ribosomes (isolated from normal rat brain cortex and hypothalamus) are treated with Δ^9-THC, very little or no change in the enzymic activity occurs.

Tables 3 and 4 summarize the results of the release of protein, RNA, and acid-soluble nucleotides from the ribosomes of Δ^9-THC-treated brain cortex and hypothalamus into different suspending media (Kihara *et al.*, 1961; Eardman *et al.*, 1968; Gillchriest and Bock, 1958) under *in vivo* and *in vitro* conditions. The results of Table 3 indicate that after treatment with a single dose of Δ^9-THC (2 mg/kg), ribosomal particles become less susceptible to breakdown, as revealed by the decreased release of protein, RNA, and acid-soluble nucleotides, than do the normal ribosomes. On the other hand, ribosomes

TABLE 3

STABILITY OF RIBOSOME FROM *IN VIVO* Δ^9-THC-TREATED RAT BRAIN CORTEX AND HYPOTHALAMUS[a]

Sources of ribosomes	Treatment	Released components	Amounts (mg/5 ml) released in 3 hr in suspension media of: Controls (in 0.005 M Mg-cacodylate buffer, pH 7.0)	NaCl (0.05 M)	KCl (0.05 M)	$MgCl_2$ (0.05 M)	Phosphate (0.05 M)	EDTA (0.01 M)	Urea (4 M)	Spermine (0.1 M)
Cortex	Control (saline–Tween 80)	Protein	0.35 ± 0.02	0.78 ± 0.04	0.50 ± 0.03	0.44 ± 0.03	0.65 ± 0.02	0.60 ± 0.05	2.17 ± 0.13	0.45 ± 0.02
		Total RNA	0.15 ± 0.02	0.35 ± 0.03	0.18 ± 0.01	0.24 ± 0.01	0.28 ± 0.01	0.25 ± 0.01	0.72 ± 0.06	0.18 ± 0.01
		Acid-soluble nucleotides	0.03 ± 0.005	0.08 ± 0.008	0.04 ± 0.004	0.04 ± 0.003	0.06 ± 0.002	0.08 ± 0.002	0.22 ± 0.03	0.04 ± 0.002
	Δ^9-THC (2 mg/kg)	Protein	0.22 ± 0.01[b]	0.61 ± 0.02[c]	0.38 ± 0.02[c]	0.30 ± 0.01[c]	0.53 ± 0.04[d]	0.46 ± 0.03[d]	2.09 ± 0.18	0.25 ± 0.02[b]
		Total RNA	0.08 ± 0.01[c]	0.28 ± 0.01[b]	0.11 ± 0.01[b]	0.17 ± 0.02[d]	0.25 ± 0.01[d]	0.18 ± 0.02[c]	0.63 ± 0.04	0.09 ± 0.002[b]
		Acid-soluble nucleotides	0.01 ± 0.00[c]	0.05 ± 0.003[b]	0.01 ± 0.00[c]	0.01 ± 0.00[b]	0.03 ± 0.002[b]	0.04 ± 0.002[b]	0.16 ± 0.01	0.008 ± 0.00[b]
	Δ^9-THC (10 mg/mg)	Protein	0.63 ± 0.04[b]	0.83 ± 0.06	0.68 ± 0.04[b]	0.64 ± 0.03[b]	0.78 ± 0.05[d]	0.82 ± 0.06[d]	2.40 ± 0.20	0.54 ± 0.06
		Total RNA	0.47 ± 0.03[b]	0.68 ± 0.04[b]	0.50 ± 0.02[b]	0.45 ± 0.03[b]	0.64 ± 0.05[b]	0.65 ± 0.04[b]	0.80 ± 0.06	0.32 ± 0.02[b]
		Acid-soluble nucleotides	0.06 ± 0.002[b]	0.08 ± 0.008	0.06 ± 0.002	0.04 ± 0.001	0.08 ± 0.005[c]	0.09 ± 0.004	0.24 ± 0.01	0.06 ± 0.005[c]
	Δ^9-THC (50 mg/kg)	Protein	0.75 ± 0.06[b]	1.08 ± 0.06[c]	0.88 ± 0.04[b]	0.68 ± 0.03[b]	0.92 ± 0.05[b]	0.90 ± 0.04[b]	2.75 ± 0.18	0.68 ± 0.03[b]
		Total RNA	0.57 ± 0.03[b]	0.64 ± 0.03[b]	0.56 ± 0.02[b]	0.42 ± 0.02[b]	0.72 ± 0.04[b]	0.78 ± 0.02[b]	0.88 ± 0.07	0.45 ± 0.02[b]
		Acid-soluble nucleotides	0.17 ± 0.01[b]	0.22 ± 0.01[b]	0.19 ± 0.01[b]	0.15 ± 0.008[b]	0.17 ± 0.009[b]	0.21 ± 0.01[b]	0.28 ± 0.01	0.13 ± 0.006[b]
	Δ^9-THC (10 mg/kg/day)[e]	Protein	0.92 ± 0.04[b]	1.25 ± 0.07[b]	0.94 ± 0.06[b]	0.88 ± 0.05[b]	1.18 ± 0.06[b]	1.12 ± 0.05[b]	3.20 ± 0.19[c]	0.88 ± 0.06[b]
		Total RNA	0.63 ± 0.03[b]	0.72 ± 0.03[b]	0.55 ± 0.04[b]	0.60 ± 0.03[b]	0.87 ± 0.04[b]	0.76 ± 0.03[b]	0.92 ± 0.07[d]	0.59 ± 0.03[b]
		Acid-soluble nucleotides	0.24 ± 0.01[b]	0.24 ± 0.012[b]	0.28 ± 0.01[b]	0.28 ± 0.02[b]	0.43 ± 0.02[b]	0.24 ± 0.02[b]	0.32 ± 0.025[d]	0.16 ± 0.008[b]

Hypothalamus	Control (saline–Tween 80)	Protein	0.49 ± 0.02	0.86 ± 0.04	0.58 ± 0.03	0.52 ± 0.03	0.76 ± 0.04	0.69 ± 0.02	2.89 ± 0.16	0.50 ± 0.04
		Total RNA	0.17 ± 0.01	0.34 ± 0.02	0.19 ± 0.01	0.22 ± 0.01	0.23 ± 0.01	0.27 ± 0.01	0.80 ± 0.03	0.18 ± 0.01
		Acid-soluble nucleotides	0.04 ± 0.003	0.07 ± 0.01	0.05 ± 0.008	0.04 ± 0.002	0.08 ± 0.004	0.07 ± 0.005	0.25 ± 0.01	0.04 ± 0.006
	Δ^9-THC (2 mg/kg)	Protein	0.40 ± 0.02[c]	0.72 ± 0.04[d]	0.48 ± 0.05	0.40 ± 0.02[d]	0.66 ± 0.05	0.62 ± 0.04[c]	2.68 ± 0.20	0.40 ± 0.02[d]
		Total RNA	0.10 ± 0.001[b]	0.26 ± 0.01[c]	0.11 ± 0.01[b]	0.16 ± 0.01[c]	0.19 ± 0.01[d]	0.21 ± 0.01[c]	0.75 ± 0.05	0.08 ± 0.004[b]
		Acid-soluble nucleotides	0.02 ± 0.003[b]	0.04 ± 0.003[d]	0.02 ± 0.001	0.01 ± 0.003[b]	0.05 ± 0.008[c]	0.06 ± 0.004	0.22 ± 0.01[d]	0.01 ± 0.00[b]
	Δ^9-THC (10 mg/kg)	Protein	0.61 ± 0.03[c]	0.93 ± 0.05	0.65 ± 0.03	0.58 ± 0.02	0.81 ± 0.04	0.75 ± 0.04[c]	2.97 ± 0.15	0.54 ± 0.02[d]
		Total RNA	0.39 ± 0.01[b]	0.50 ± 0.02[b]	0.28 ± 0.01[b]	0.39 ± 0.01[b]	0.45 ± 0.03[b]	0.54 ± 0.03[b]	0.98 ± 0.04[c]	0.32 ± 0.01[b]
		Acid-soluble nucleotides	0.05 ± 0.001[c]	0.08 ± 0.002	0.06 ± 0.002	0.06 ± 0.003[b]	0.10 ± 0.01	0.09 ± 0.004[c]	0.27 ± 0.01	0.06 ± 0.005[d]
	Δ^9-THC (50 mg/kg)	Protein	0.77 ± 0.04[b]	1.03 ± 0.07[d]	0.78 ± 0.05[c]	0.71 ± 0.04[c]	0.94 ± 0.07[d]	0.88 ± 0.06[d]	3.01 ± 0.17[d]	0.68 ± 0.04[b]
		Total RNA	0.48 ± 0.02[b]	0.58 ± 0.03[b]	0.47 ± 0.03[b]	0.48 ± 0.02[b]	0.55 ± 0.03[b]	0.60 ± 0.05[b]	0.99 ± 0.06[c]	0.45 ± 0.02[b]
		Acid-soluble nucleotides	0.17 ± 0.01[b]	0.20 ± 0.02[b]	0.16 ± 0.01[b]	0.15 ± 0.01[b]	0.23 ± 0.13[b]	0.18 ± 0.01[b]	0.32 ± 0.02[d]	0.15 ± 0.01[b]
	Δ^9-THC (10 mg/kg/day)	Protein	0.98 ± 0.05[b]	1.17 ± 0.07[b]	0.90 ± 0.04[b]	0.83 ± 0.04[b]	1.06 ± 0.07[c]	1.05 ± 0.06[c]	3.29 ± 0.19[c]	0.88 ± 0.04[b]
		Total RNA	0.57 ± 0.02[b]	0.63 ± 0.03[b]	0.58 ± 0.02[b]	0.57 ± 0.03[b]	0.60 ± 0.03[b]	0.70 ± 0.03[c]	1.12 ± 0.09[b]	0.54 ± 0.03[b]
		Acid-soluble nucleotides	0.17 ± 0.01[b]	0.20 ± 0.01[b]	0.22 ± 0.01[b]	0.24 ± 0.02[b]	0.30 ± 0.02[b]	0.20 ± 0.01[c]	0.33 ± 0.02[d]	0.17 ± 0.01[b]

[a] Results expressed as mean ± SE of six different experiments. A 25-mg portion of ribosomes (containing 16.1 mg of protein and 8.9 mg of RNA) was suspended in 5 ml of 0.005 M Mg-cacodylate buffer, pH 7.0, in which the agents indicated in the Table were dissolved with an adjustment of pH wherever necessary. After incubation at 37°C for 3 hr, the mixture was centrifuged at 105,000*g* for 1 hr. The supernatants were used for the estimation of proteins, RNA[6], and acid-soluble nucleotides. The concentration of acid-soluble nucleotides was determined at an absorbance value of 33 at 260 nm of a solution containing 1 mg equivalent of nucleotide mixture obtained from the complete hydrolysis of 1 mg of yeast RNA.

[b] Significantly different from control; $p < 0.001$, Student's t test.

[c] $p < 0.005$.

[d] $p < 0.05$.

[e] Treatment was continued for 21 consecutive days.

TABLE 4

STABILITY OF RIBOSOMES FROM *IN VITRO* Δ^9-THC-TREATED RAT BRAIN CORTEX AND HYPOTHALAMUS SLICES

Sources of ribosomes	Treatment	Released components	Amounts (mg/5 ml) released in 3 hr in suspension media of: Control (in 0.005 M Mg-cacodylate buffer, pH 7.0)	NaCl (0.05 M)	KCl (0.05 M)	$MgCl_2$ (0.05 M)	Phosphate (0.05 M)	EDTA (0.01 M)	Urea (4 M)	Spermine (0.1 mM)
Cortex	Control (saline–Tween 80)	Protein	0.36 ± 0.02	0.75 ± 0.04	0.51 ± 0.03	0.42 ± 0.02	0.67 ± 0.03	0.60 ± 0.02	2.09 ± 0.13	0.40 ± 0.02
		Total RNA	0.14 ± 0.01	0.36 ± 0.01	0.19 ± 0.01	0.22 ± 0.01	0.29 ± 0.02	0.23 ± 0.01	0.70 ± 0.04	0.16 ± 0.01
		Acid-soluble nucleotides	0.03 ± 0.002	0.07 ± 0.003	0.03 ± 0.002	0.03 ± 0.003	0.07 ± 0.004	0.23 ± 0.03	0.26 ± 0.01	0.04 ± 0.002
	Δ^9-THC (0.2 mg/g of tissue slices)	Protein	0.10 ± 0.002[b]	0.58 ± 0.02[c]	0.27 ± 0.01[b]	0.16 ± 0.008[b]	0.45 ± 0.03[c]	0.43 ± 0.02[c]	1.88 ± 0.08[d]	0.14 ± 0.01[b]
		Total RNA	0.06 ± 0.003[b]	0.32 ± 0.01[b]	0.14 ± 0.01[c]	0.08 ± 0.003[b]	0.23 ± 0.01[d]	0.17 ± 0.01[c]	0.60 ± 0.04[d]	0.07 ± 0.004[b]
		Acid-soluble nucleotides	0.006 ± 0.00[b]	0.04 ± 0.002[b]	0.01 ± 0.00[b]	0.02 ± 0.00[d]	0.05 ± 0.002[b]	0.06 ± 0.003[d]	0.16 ± 0.01[d]	0.01 ± 0.00[b]
	Δ^9-THC (0.5 mg/g of tissue slices)	Protein	0.19 ± 0.01[b]	0.72 ± 0.05	0.30 ± 0.01[b]	0.24 ± 0.01[b]	0.52 ± 0.02[c]	0.45 ± 0.15[b]	1.96 ± 0.08	0.38 ± 0.02
		Total RNA	0.10 ± 0.005[c]	0.28 ± 0.01[c]	0.10 ± 0.003[b]	0.19 ± 0.008[d]	0.26 ± 0.01	0.22 ± 0.01	0.65 ± 0.03	0.14 ± 0.01
		Acid-soluble nucleotides	0.01 ± 0.00	0.04 ± 0.002[b]	0.02 ± 0.00[b]	0.02 ± 0.002[d]	0.05 ± 0.004[c]	0.05 ± 0.003[b]	0.20 ± 0.01[c]	0.03 ± 0.002[c]
	Δ^9-THC (1.0 mg/g of tissue slices)	Protein	0.60 ± 0.04[b]	1.12 ± 0.06[c]	0.86 ± 0.04[b]	0.61 ± 0.03[b]	0.94 ± 0.04[b]	0.97 ± 0.05[b]	2.67 ± 0.16[b]	0.50 ± 0.03[d]
		Total RNA	0.37 ± 0.02[c]	0.54 ± 0.03[c]	0.54 ± 0.02[b]	0.40 ± 0.02[c]	0.81 ± 0.04[b]	0.86 ± 0.05[b]	0.90 ± 0.05[c]	0.30 ± 0.01[b]
		Acid-soluble nucleotides	0.05 ± 0.005[c]	0.12 ± 0.01[d]	0.10 ± 0.01[b]	0.08 ± 0.004[c]	0.15 ± 0.01[b]	0.17 ± 0.01[b]	0.29 ± 0.012	0.04 ± 0.002

	Δ^9-THC (5.0 mg/g of tissue slices)	Protein	0.83 ± 0.04[b]	1.35 ± 0.07[b]	0.88 ± 0.03[b]	0.82 ± 0.03[b]	1.19 ± 0.08[b]	1.17 ± 0.09[b]	3.27 ± 0.21[b]	0.71 ± 0.03[b]
		Total RNA	0.58 ± 0.03[b]	0.76 ± 0.03[b]	0.56 ± 0.02[b]	0.54 ± 0.02[b]	0.92 ± 0.05[b]	0.81 ± 0.04[b]	0.98 ± 0.04[b]	0.46 ± 0.02[b]
		Acid-soluble nucleotides	0.12 ± 0.01[b]	0.20 ± 0.01[b]	0.14 ± 0.01[b]	0.13 ± 0.01[b]	0.23 ± 0.01[b]	0.18 ± 0.01[b]	0.29 ± 0.01	0.07 ± 0.004[b]
Hypothalamus	Control (saline–Tween 80)	Protein	0.48 ± 0.03	0.87 ± 0.04	0.58 ± 0.02	0.56 ± 0.03	0.75 ± 0.04	0.67 ± 0.03	2.79 ± 0.16	0.49 ± 0.02
		Total RNA	0.17 ± 0.01	0.33 ± 0.01	0.21 ± 0.01	0.24 ± 0.01	0.25 ± 0.01	0.26 ± 0.01	0.70 ± 0.04	0.16 ± 0.01
		Acid-soluble nucleotides	0.04 ± 0.002	0.06 ± 0.003	0.05 ± 0.002	0.04 ± 0.003	0.06 ± 0.002	0.06 ± 0.003	0.26 ± 0.01	0.04 ± 0.003
	Δ^9-THC (0.2 mg/g of tissue slices)	Protein	0.28 ± 0.01[b]	0.60 ± 0.04[b]	0.32 ± 0.02[c]	0.26 ± 0.01[b]	0.49 ± 0.02[b]	0.39 ± 0.02[b]	2.40 ± 0.15[c]	0.23 ± 0.01[b]
		Total RNA	0.10 ± 0.01[b]	0.23 ± 0.01[c]	0.15 ± 0.01[c]	0.12 ± 0.01[b]	0.19 ± 0.01[c]	0.19 ± 0.01[c]	0.64 ± 0.03	0.08 ± 0.003[b]
		Acid-soluble nucleotides	0.01 ± 0.00[b]	0.04 ± 0.002[d]	0.03 ± 0.002[d]	0.02 ± 0.00[c]	0.03 ± 0.002[c]	0.04 ± 0.002[d]	0.21 ± 0.01[c]	0.02 ± 0.00[c]
	Δ^9-THC (0.5 mg/g of tissue slices)	Protein	0.38 ± 0.02[d]	0.72 ± 0.04[d]	0.44 ± 0.02[b]	0.39 ± 0.02[b]	0.58 ± 0.03[c]	0.51 ± 0.03[c]	2.68 ± 0.18	0.38 ± 0.02[c]
		Total RNA	0.13 ± 0.01[d]	0.30 ± 0.02	0.18 ± 0.01[d]	0.20 ± 0.01[c]	0.22 ± 0.01	0.23 ± 0.01[d]	0.62 ± 0.04	0.10 ± 0.01[c]
		Acid-soluble nucleotides	0.03 ± 0.003[d]	0.03 ± 0.002[b]	0.03 ± 0.002[b]	0.01 ± 0.00[b]	0.04 ± 0.003[b]	0.04 ± 0.002[b]	0.19 ± 0.01[b]	0.15 ± 0.00[b]
	Δ^9-THC (1.0 mg/g of tissue slices)	Protein	0.78 ± 0.04[b]	1.21 ± 0.07[b]	0.75 ± 0.05[c]	0.64 ± 0.03[c]	0.99 ± 0.06[b]	0.98 ± 0.06[b]	3.21 ± 0.20[c]	0.56 ± 0.03[d]
		Total RNA	0.27 ± 0.01[b]	0.47 ± 0.02[b]	0.43 ± 0.02[b]	0.32 ± 0.01[b]	0.44 ± 0.02[b]	0.39 ± 0.02[b]	0.86 ± 0.05[d]	0.25 ± 0.01[b]
		Acid-soluble nucleotides	0.08 ± 0.004[c]	0.11 ± 0.01[c]	0.07 ± 0.003[c]	0.06 ± 0.002[b]	0.12 ± 0.01[c]	0.13 ± 0.01[c]	0.28 ± 0.01	0.05 ± 0.002
	Δ^9-THC (5.0 mg/g of total slices)	Protein	1.08 ± 0.06[b]	1.49 ± 0.07[b]	1.28 ± 0.08[b]	1.38 ± 0.09[b]	1.29 ± 0.08[b]	1.35 ± 0.10[b]	3.46 ± 0.21[b]	0.82 ± 0.04[b]
		Total RNA	0.48 ± 0.02[b]	0.52 ± 0.03[b]	0.46 ± 0.02[b]	0.40 ± 0.02[b]	0.68 ± 0.03[b]	0.64 ± 0.03[b]	0.90 ± 0.04[c]	0.38 ± 0.01[b]
		Acid-soluble nucleotides	0.16 ± 0.01[b]	0.16 ± 0.01[b]	0.13 ± 0.008[b]	0.11 ± 0.007[b]	0.17 ± 0.01[c]	0.18 ± 0.01[b]	0.30 ± 0.01[d]	0.08 ± 0.05[c]

[a] Results expressed as mean ± SE of six different experiments. A 25-mg portion of ribosomes (containing 16.1 mg of protein and 8.9 mg RNA) was suspended in 0.005 M Mg-cacodylate buffer, pH 7.0. The other conditions used were the same as in Table 5. Conditions used for *in vitro* treatment of rat brain cortex and hypothalamic slices with Δ^9-THC are described in detail under Methods.

[b] Significantly different from control treatment; $p < 0.001$, Student's t test.

[c] $p < 0.005$.

[d] $p < 0.05$.

TABLE 5

In Vivo AND *In Vitro* EFFECT OF Δ^9-THC ON HYPERCHROMIC VALUES OF RIBOSOMES AND EXTRACTED RNAs FROM RAT BRAIN CORTEX AND HYPOTHALAMUS[a]

Condition of treatment	Source of ribosomes and rRNA	*In vivo* On hydrolysis by 0.3 M KOH solution for 18 hr at 37°C followed by removal of proteins and neutralization (dissolved in 0.014 M acetate Veronal buffer, pH 7.0)		On degradation by pancreatic RNase in 0.014 M acetate veronal buffer, pH 7.5, protein was removed and hydrolysate was neutralized (dissolved in 0.014 M acetate veronal buffer, pH 7.0)		On heating in 0.028 M acetate veronal buffer, pH 7.0, for 20 min at 85°C in stoppered cuvettes in a thermostatic heating chamber attached to Beckman DU spectrophotometer	
		Ribosomes	rRNA	Ribosomes	rRNA	Ribosomes	rRNA
In vivo							
Control (saline–Tween 80)	Brain cortex	33.5 ± 1.0	30.7 ± 1.1	23.8 + 0.6	24.3 ± 0.6	14.0 ± 0.2	12.8 + 0.2
	Hypothalamus	28.9 ± 1.5	30.1 ± 1.0	22.6 ± 0.7	23.8 ± 0.8	16.1 ± 0.3	16.8 ± 0.2
Δ^9-THC (2 mg/kg)	Brain cortex	33.5 ± 0.9	32.5 ± 1.0	24.5 ± 1.0	25.6 ± 0.9	16.0 ± 0.1	15.4 ± 0.2
	Hypothalamus	28.2 ± 1.0	29.0 ± 0.7	26.1 ± 0.9	25.7 ± 1.0	20.0 ± 0.2	19.5 ± 0.3
Δ^9-THC (10 mg/kg)	Brain cortex	31.7 ± 1.2	32.5 ± 1.1	24.1 ± 0.8	23.7 ± 0.9	9.2 ± 0.2	9.8 ± 0.1

	Hypothalamus	28.4 ± 0.9	30.7 ± 1.1	24.0 ± 0.07	22.9 ± 0.5	13.8 ± 0.2	14.2 ± 0.1
Δ^9-THC (50 mg/kg)	Brain cortex	31.4 ± 1.0	33.1 ± 1.0	24.8 ± 0.8	25.6 ± 1.0	8.3 ± 0.1	7.7 ± 0.2
	Hypothalamus	30.7 ± 1.1	30.6 ± 0.7	25.7 ± 1.0	24.1 ± 1.0	11.4 ± 0.1	12.0 ± 0.3
Δ^9-THC (10 mg/kg/day)	Brain cortex	33.5 ± 1.1	32.6 ± 0.8	24.0 ± 1.0	25.1 ± 0.7	7.4 ± 0.2	7.2 ± 0.3
	Hypothalamus	31.1 ± 0.8	30.3 ± 0.5	23.9 ± 0.5	23.6 ± 0.8	9.6 ± 0.2	10.2 ± 0.1
In vitro							
Control (saline–Tween 80)	Brain cortex	32.0 ± 0.8	33.7 ± 0.9	24.0 ± 1.1	25.4 ± 0.7	13.4 ± 0.3	13.6 ± 0.2
	Hypothalamus	28.9 ± 1.0	30.1 ± 1.0	22.7 ± 0.7	22.5 ± 1.0	16.5 ± 0.2	17.0 ± 0.1
Δ^9-THC (2 mg/g of	Brain cortex	30.1 ± 1.2	31.2 ± 0.8	23.8 ± 1.0	25.1 ± 0.8	16.2 ± 0.2	16.0 ± 0.3
tissue slices)	Hypothalamus	31.6 ± 0.7	32.0 ± 0.9	23.0 ± 0.9	22.0 ± 0.5	19.1 ± 0.4	19.6 ± 0.2
Δ^9-THC (0.5 mg/g of	Brain cortex	31.0 ± 0.9	34.0 ± 1.0	23.9 ± 0.9	26.0 ± 0.1	15.0 ± 0.1	15.2 ± 0.1
tissue slices)	Hypothalamus	33.2 ± 0.8	33.1 ± 0.7	23.7 ± 1.0	24.0 ± 1.0	18.3 ± 0.2	18.9 ± 0.2
Δ^9-THC (1.0 mg/g of	Brain cortex	33.5 ± 0.7	31.7 ± 1.0	24.7 ± 0.9	23.9 ± 0.6	10.9 ± 0.2	11.6 ± 0.2
tissue slices)	Hypothalamus	34.0 ± 1.1	33.1 ± 0.6	22.9 ± 0.4	22.1 ± 1.0	13.8 ± 0.2	14.8 ± 0.2
Δ^9-THC (5.0 mg/g of •	Brain cortex	32.5 ± 0.5	33.9 ± 1.0	22.8 ± 0.9	23.0 ± 1.1	7.3 ± 0.1	7.5 ± 0.2
tissue slices)	Hypothalamus	33.0 ± 0.9	30.9 ± 1.1	23.4 ± 0.7	24.1 ± 1.0	10.4 ± 0.2	10.8 ± 0.3

[a] Mean of eight determinations using different ribosomes and rRNA preparations ± SE.
[b] Treatment was continued for 21 consecutive days.

isolated from the same tissues of rats treated with Δ^9-THC at high doses (10 and 50 mg/kg) and under chronic conditions (10 mg/kg-day for 21 days) become more susceptible to release of protein, RNA, and acid-soluble nucleotides than do those of normal rat tissues. Incubation in spermine-containing medium (Table 3, last column) significantly decreased the release of ribosomal components from the ribosomes of Δ^9-THC-treated tissues compared with the release of those components in other experimental media. However, this release in spermine-containing medium at high doses and under chronic conditions of treatment is quantitatively higher than from the ribosomes of control brain cortex and hypothalamus.

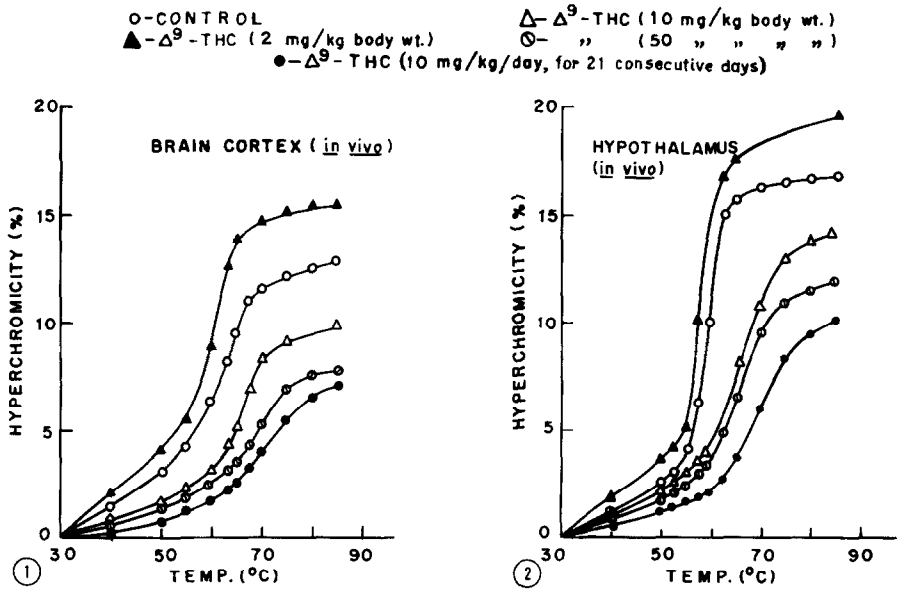

FIG. 1. Thermal hyperchromicity of rRNAs of brain cortex of control (saline–Tween 80) and Δ^9-THC-treated rats. Each point represents the average of six different determinations.

FIG. 2. Thermal hyperchromicity of rRNAs of hypothalamus of control (saline–Tween 80) and Δ^9-THC-treated rats. Each point represents the average of six different determinations.

Table 4 indicates that, like *in vivo* treatment, *in vitro* treatment of rat brain cortex and hypothalamic slices with Δ^9-THC stabilizes the ribosomes at low doses (0.2 and 0.5 mg/g of tissue slices). However Δ^9-THC at high doses (1.0 and 5.0 mg/g of tissue slices) produces instability in ribosomes from the same tissues.

Table 5 shows the *in vivo* and *in vitro* effects of Δ^9-THC on hyperchromic values of the ribosomes and their extracted rRNAs from brain cortex and hypothalamus as estimated by alkaline hydrolysis, by treatment with pancreatic RNase, and after heating. These results suggest that these hyperchromic values from ribosomes of brain cortex and hypothalamus and their corresponding extracted RNAs are very similar.

From Figs. 1–4 it is evident that the melting temperature (T_m) of the rRNA preparations form brain cortex and hypothalamus of control and Δ^9-THC treatment (under both *in vivo* and *in vitro* conditions) lies between 55 and 65°C. It is also noted that the thermal profile curves of total rRNAs from brain cortex and hypothalamus at

low doses of Δ^9-THC treatment, under *in vivo* (2 mg/kg) and *in vitro* (0.2 and 0.5 mg/g of wet tissue slices) conditions, are comparatively steeper than those of corresponding rRNAs of control tissue; at higher doses (10 and 50 mg/kg under *in vivo* condition; 1.0 and 5.0 mg/g of wet tissue slices under *in vitro* condition) and during chronic treatment with Δ^9-THC (10 mg/kg/day for 21 days) the curves are comparatively less steep than those of corresponding rRNAs of control tissue. The steepness of the transition (increased slopes) which is observed in the rRNA preparations from Δ^9-THC-treated (at low doses) brain cortex and hypothalamus (under both *in vivo* and *in vitro* conditions) indicates that Δ^9-THC at low doses increased the hydrogen-bonded

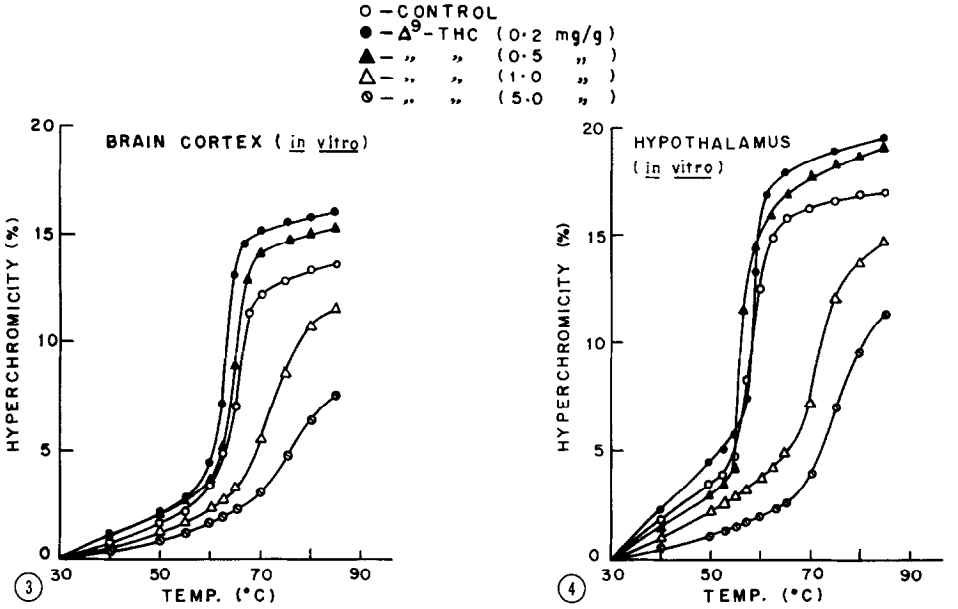

FIG. 4. Thermal hyperchromicity of rRNAs of control (saline–Tween 80) and Δ^9-THC-treated (under *in vitro* condition) rat brain hypothalamic slices. Each point represents the average of six different determinations.

FIG. 3. Thermal hyperchromicity of rRNAs of control (saline–Tween 80) and Δ^9-THC-treated (under *in vitro* condition) rat brain cortex slices. Each point represents the average of six different determinations.

structure of rRNAs of brain cortex and hypothalamus. At high doses (under *in vivo* and *in vitro* conditions) and during chronic treatment with Δ^9-THC, on the other hand, the slopes of the melting temperature curve of control rRNA preparations are reduced. These results indicate that part of the hydrogen-bonded structure of rRNAs of brain cortex and hypothalamus, at high doses and during chronic treatment with Δ^9-THC, has been lost.

Assuming that the percentage of thermal hyperchromicity between 30 and 85°C for total rRNA in control tissues is indicative of 100% hydrogen-bonded structure, the rRNAs of brain cortex and hypothalamus, expressed to low doses of Δ^9-THC, have 113–120 and 111–116% hydrogen-bonded structure, respectively. At high doses and during chronic treatment with Δ^9-THC, the hydrogen bonding of brain cortex and hypothalamic total rRNAs become 55–84 and 56–87% respectively.

It is evident from Figs. 5–8 that the rRNA preparations of both brain cortex and hypothalamus treated with Δ^9-THC at low doses, under *in vivo* (2 mg/kg) and *in vitro* (0.2 and 0.5 mg/g of wet tissue slices) conditions, show markedly lower formaldehyde hyperchromicity at 30°C than those of corresponding total rRNAs of control tissues; at high doses, under *in vivo* (10 and 50 mg/kg) and *in vitro* (1.0 and 5.0 mg/g of wet tissue slices) conditions, and during chronic treatment (10 mg/kg/day for 21 days), these preparations show strikingly greater formaldehyde hyperchromicity at 30°C than those

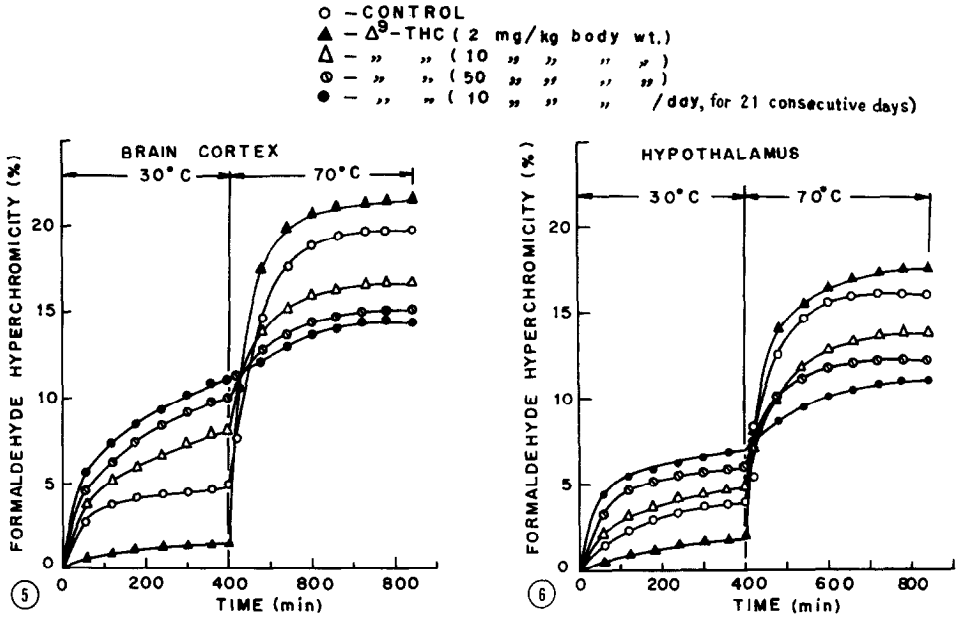

FIG. 5. Formaldehyde hyperchromicity of rRNAs of brain cortex of control (saline–Tween 80) and Δ^9-THC-treated (*in vivo*) rats. Each point represents the average of six different determinations.

FIG. 6. Formaldehyde hyperchromicity of rRNAs of hypothalamus of control (saline–Tween 80) and Δ^9-THC-treated (*in vivo*) rats. Each point represents the average of six different determinations.

of corresponding total rRNAs of control brain tissues. At 70°C the total rRNAs of brain cortex and hypothalamus treated with Δ^9-THC at low doses, under both *in vivo* and *in vitro* conditions, exhibits a greater hyperchromic effect than the corresponding vehicle-treated (control) brain cortex and hypothalamic total rRNA; whereas at higher doses (under *in vivo* and *in vitro* conditions) and during chronic treatment with Δ^9-THC the rRNAs of both brain cortex and hypothalamus exhibit a lower hyperchromic effect than the corresponding vehicle-treated brain tissues.

On the basis of the assumption that the formaldehyde hyperchromic effect at 30°C represents the extent of nonhydrogen-bonded structure and the formaldehyde effect at 70°C represents the extent of hydrogen-bonded structure in RNA, it is possible to estimate the percentage of both nonhydrogen-bonded and hydrogen-bonded structures present in the rRNA molecules. It is estimated that as a result of the *in vivo* action of Δ^9-THC: (a) 18 and 15% of the hydrogen-bonded structure in total rRNAs of brain cortex and hypothalamus, respectively, are increased due to low doses of Δ^9-THC (2 mg/kg); and (b) 25–49 and 10–38% of the hydrogen-bonded structure in total rRNA of

brain cortex and hypothalamus, respectively, appear to be lost due to high doses (10 and 50 mg/kg) and also as a result of chronic treatment with Δ^9-THC (10 mg/kg/day for 21 days). Like *in vivo* treatment, *in vitro* treatment with Δ^9-THC at low levels increases the hydrogen-bonded structure of total rRNA of brain cortex (13–16%) and hypothalamus (11–14%), using 0.2 and 0.5 mg/g of wet tissue slices; high levels decrease the hydrogen-bonded structure in total rRNA of brain cortex (18–42%) and hypothalamus (10–28%), using 1.0 and 5.0 mg/g of wet tissue slices.

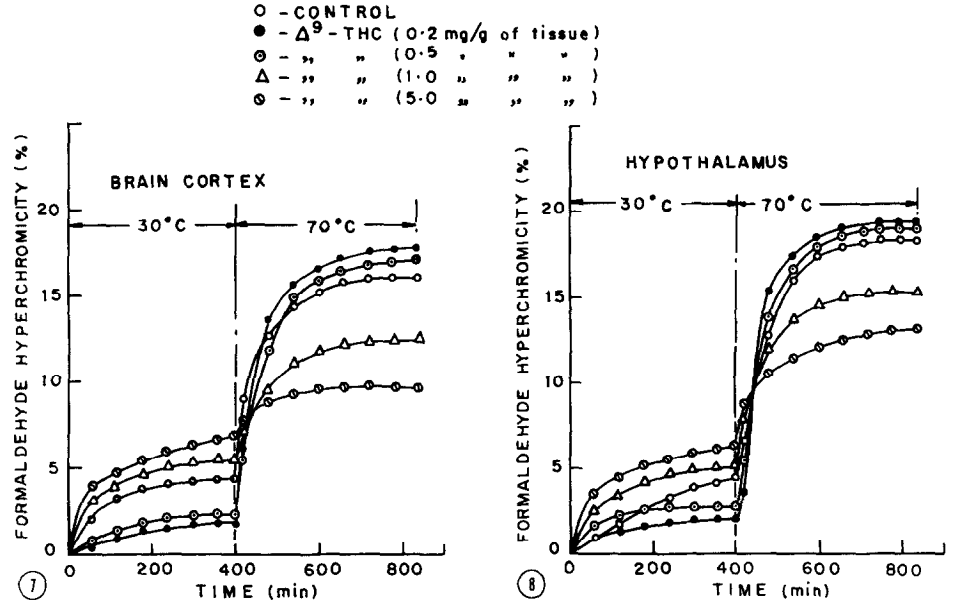

FIG. 7. Formaldehyde hyperchromicity of rRNAs of control (saline–Tween 80) and Δ^9-THC-treated (under *in vitro* condition) rat brain cortex slices. Each point represents the average of six different determinations.

FIG. 8. Formaldehyde hyperchromicity of rRNAs of control (saline–Tween 80) and Δ^9-THC-treated (under *in vitro* condition) rat brain hypothalamic slices. Each point represents the average of six different determinations.

DISCUSSION

The results of the present study indicate that Δ^9-THC exerts a characteristic dose-dependent response on the stability of rat brain cortex and hypothalamic ribosomes under both *in vivo* and *in vitro* conditions of treatment. The enzymes RNase, PMase, and PDase are integral parts of brain ribosomes and are known to be involved in the depolymerization of RNA and other polyribonucleotides. Significant stimulation or inhibition of the specific activities of these three enzymes depending on the concentrations of Δ^9-THC and conditions of treatment reflect specific changes in the brain ribosomal profile. However, it appears that the observed changes in activities of these enzyme systems may not be due to direct action of Δ^9-THC on the ribosomal particles, as very little change in the specific activities of the enzymes has been observed when pure ribosomal preparations of either brain cortex or hypothalamus are treated with Δ^9-

THC. Results of the stability studies of brain ribosomes, measured in terms of the release of proteins, RNA, and acid-soluble nucleotides in different suspension media (Tables 3 and 4), indicate however that the macromolecular structure of ribosomes is affected by Δ^9-THC treatment. Once structural change has occurred, it cannot be completely reversed by spermine, although the breakdown of ribosomal components under these conditions may be decreased to some extent. Δ^9-THC induced stability at low doses and instability at high doses and also under chronic conditions of treatment of the brain cortex and hypothalamic ribosomes may perhaps account for the observed stimulation or depression of specific activities of the ribosomal enzymes.

The use of thermal and formaldehyde hyperchromic effects in the present study for quantitative assessment of the degree of hydrogen-bonded structure in rRNAs of brain cortex and hypothalamus, is based on principles and assumptions which are quite arbitrary. Hence the degree of hydrogen-bonded structure of macromolecules estimated independently by thermal hyperchromic effect and by formaldehyde reaction rate may not quantitatively agree with each other, although some qualitative agreement should be observed. In the present study, the hyperchromic effect, as observed by the two methods, has given qualitatively similar results concerning the relative degree of hydrogen-bonded structure of total rRNAs under investigation. In the present investigation, it has been assumed that the state of hydrogen bonding of RNA in ribosomes is more or less retained in the extracted RNAs, since the hyperchromicity values of brain cortex and hypothalamic ribosomes and their extracted RNAs as determined by alkaline hydrolysis or by treatment with ribonuclease are quite similar in the present results (Table 5). Comparison of the thermal hyperchromicity and formaldehyde reactivity of RNA preparations from control and Δ^9-THC-treated tissues is possible, since the ribosomal RNA preparations have almost identical chemical composition and uv absorption characteristics as observed under the present conditions of treatment.

So, it may be inferred that the observed changes in the degree of hydrogen-bonded structure of total rRNA of brain cortex and hypothalamus, as measured by thermal and formaldehyde hyperchromicity values (Figs. 1–7), might have resulted from the action of Δ^9-THC. Further, the changes during chronic Δ^9-THC treatment on the degree of hydrogen bonding of rRNAs of brain cortex and hypothalamus may be due to the polar metabolites of Δ^9-THC, since more polar metabolites are produced during chronic Δ^9-THC treatment (Lemberger *et al.*, 1970, 1971).

It is well known that the stability of macromolecules depends on: (a) the availability of magnesium at the binding sites of ribosomes which also depends on (i) the counter ion concentration of Mg^{2+}, (ii) the Na^+ concentration, and (iii) phosphate ions; (b) the ionic strength of the medium; (c) the strength of the hydrogen-bonding forces; (d) the association of dissociation of the protein component from the RNA moiety; and (e) the concentration of polyamines and other basic proteins (McQuillen, 1961; Shigera and Chargaff, 1960; Ts'O Vinograd, 1961; Eardman *et al.*, 1968). In the light of these observations on the mechanism controlling the ribonucleoprotein stability, it may be speculated from the present study as well as from the knowledge of the crystal structure of Δ^9-THC (Rosenqvist and Ottersen, 1975) that Δ^9-THC induced stability of ribosomal particles of both rat brain cortex and hypothalamus at low doses of the drug (under both *in vivo* and *in vitro* conditions) may arise due to the fact that the molecules of Δ^9-THC in very low concentration may remain in the single monomeric form (Fig. 9a), and

hence the pentyl side chain which is responsible for strong hydrophobic interaction may interact with the hydrophobic regions of the ribosomal component. Furthermore, it may be possible that the planar part of the drug molecule, i.e., the phenyl ring of Δ^9-THC, is intercalated between the bases of the RNA segments having localized helical regions (Fig 10a) and produces greater stability of ribosomal RNA of both rat brain cortex and

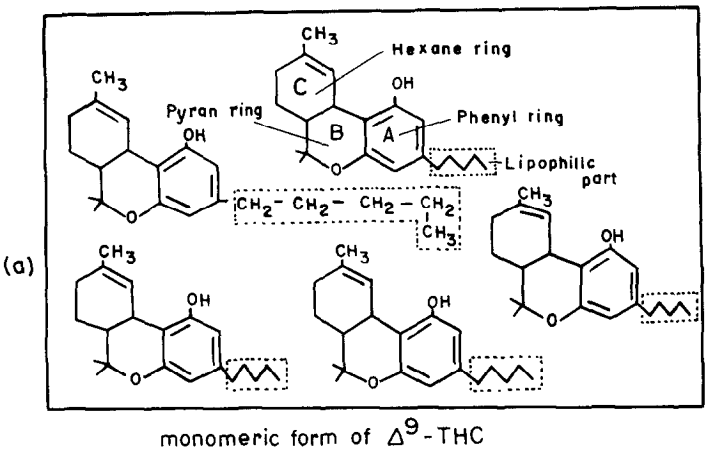

FIG. 9. Diagrammatic representation of Δ^9-THC in solution as (a) low concentration (monomeric form) and (b) high concentration (micelle formation).

hypothalamus. The instability of brain cortex and hypothalamic ribosomes at high doses (under both *in vivo* and *in vitro* conditions) and during chronic treatment with Δ^9-THC may arise as a result of the disturbance in the equilibrium between hydrogen bonding and electrostatic repulsion. At high concentrations of Δ^9-THC, the hydrophobic pentyl side chain may lead to micelle formation (Fig. 9b), as suggested by Garrett and Hunt (1974), with resultant failure to interact with ribosomal protein hydrophobically. Perhaps, in that case, it functions as a detergent to destabilize the structure of ribonucleoprotein. The destabilization may also be due to the formation of hydrogen bonding between the hydroxyl group of Δ^9-THC or its metabolites and sugar

oxygen of the RNA, as suggested by Smythies (1973). Further, it is not unlikely that at high concentration or during chronic treatment with Δ^9-THC the whole process is initiated or triggered through either the displacement of Mg^{2+} or other positively charged particles, e.g., polyamines or basic protein, by the hydroxyl group of Δ^9-THC (Fig. 10b) with the result that the intrachain hydrogen bonding in rRNA is weakened and the secondary structure of rRNA is destroyed.

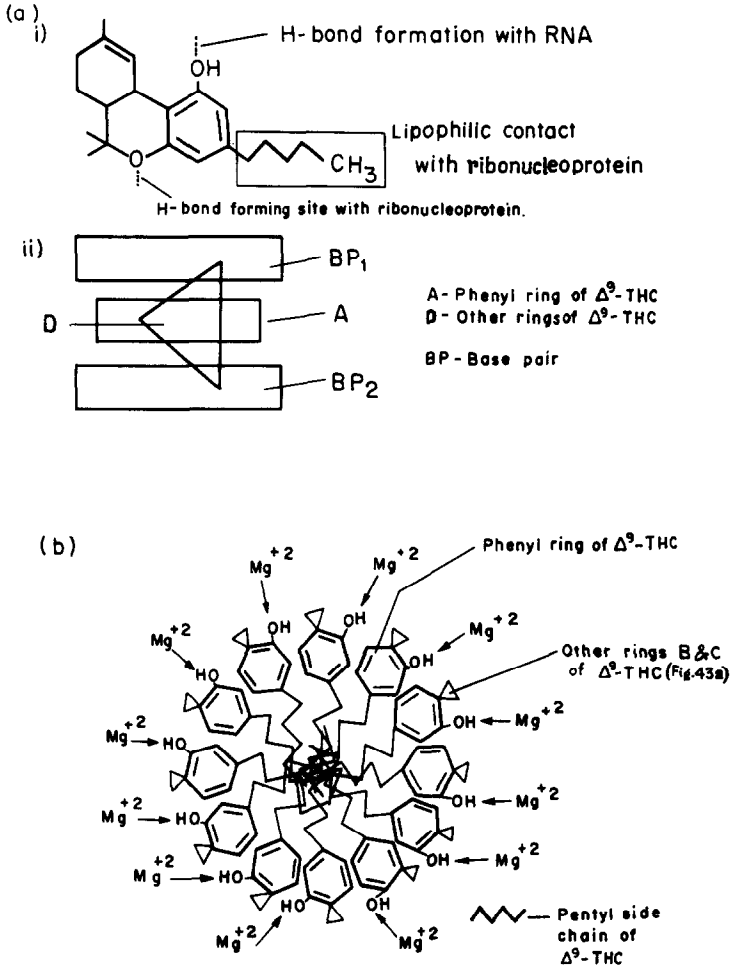

FIG. 10. Postulated binding of Δ^9-THC (a) at low concentration and (b) at high concentration with rRNA, protein, and other positively charged materials. At high concentration of Δ^9-THC Mg^{2+} or other positively charged materials may be removed.

In the present study, it has also been noted that the changes (either increase or decrease depending on the concentration and condition of treatment with Δ^9-THC) in hydrogen-bonded structure of rRNA of Δ^9-THC-treated hypothalamus are comparatively smaller than those observed in brain cortex rRNA under similar conditions of treatment. This may be due to the decreased sensitivity of hypothalamic ribosomes, in comparison to brain cortex ribosomes, toward Δ^9-THC action.

Although there is no known relevance of the observed changes of hydrogen-bonded structures of brain rRNA to the pharmacological effect of Δ^9-THC under experimental conditions, the previous observations of the effects of convulsant, CNS-stimulant, and psychotomimetic drugs on the stability of ribosomal particles (Datta and Ghosh, 1963, 1964, 1965, 1970; Ghosh *et al.*, 1965), together with the present observations, appear to suggest a relationship between the structural integrity of the RNA molecules of brain cells and the neurotropic effect of Δ^9-THC. This effect also appears to be influenced by several factors, viz., area of the brain involved, dosage of the drug used, distribution of the drug and its metabolites and duration of exposure to the drug.

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