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Mescaline-Induced Changes of Brain-Cortex Ribosomes

EFFECT OF MESCALINE ON THE HYDROGEN-BONDED STRUCTURE OF RIBONUCLEIC ACID OF BRAIN-CORTEX RIBOSOMES

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1. The action of mescaline sulphate on the hydrogen-bonded structure of the RNA constituent of ribosomes of goat brain-cortex slices was studied by using the hyperchromic effect of heating and formaldehyde reaction. 2. The ribosomal total RNA species of the mescaline-treated brain-cortex slices have a smaller proportion of hydrogen-bonded structure than the ribosomal RNA species of the untreated brain-cortex slices. 3. Mescaline also appears to have affected this lowering of hydrogen-bonded structure of the ribosomal 28S RNA of brain-cortex tissue.

In the preceding paper (Datta & Ghosh, 1970) it was reported that when brain-cortex slices are treated with mescaline sulphate (3,5,6-trimethoxyphenethylamine sulphate) the ribosomal particles become more susceptible to breakdown releasing protein, RNA, acid-soluble nucleotides and amino acids. Similar effects were previously observed during the action of convulsant and antidepressant drugs on brain-cortex slices (Datta & Ghosh, 1963*a*, 1965; Ghosh, Datta & Bhattacharyya, 1965). Since secondary structure plays a pivotal role in maintaining the stability or integrity of both RNA and protein, an attempt was made in the present study to examine the molecular mechanism of susceptibility or disorganization of RNA of ribosomal particles isolated from brain-cortex slices previously treated with mescaline.

It is well established that the organized structure of RNA is maintained mostly by hydrogen-bonding between the nitrogen and carbonyl oxygen atoms of the base-pairs, and the organized structure collapses when the hydrogen-bonds are disrupted, resulting in the exposure of free amino groups. The present study of the secondary structure involving hydrogen bonds of rRNA is based on the principles of hyperchromicity due to heating at elevated temperatures and to reaction of RNA with formaldehyde. Doty, Boedtker, Fresco, Hall & Haselkorn (1959) have found that changes in E_{260} of an RNA solution on heating have their origin in the thermal dissociation of the hydrogen-bonded base-pairs and have suggested that the thermal hyperchromicity effect may be used to determine quantitatively the percentage of hydrogen-bonded pairs or the organized structure in the helical configuration. In the present study the degree of thermal hyperchromicity at 260nm between 30 and 85°C for a solution of RNA has been taken to be approximately equivalent to the degree of hydrogen-bonded structure. On this basis the decrease in the hydrogen-bonding of the RNA constituent of ribosomes due to mescaline-treatment of brain-cortex slices was calculated.

Among a variety of methods usually applied to study the extent, size and stability of the organized helical regions of RNA, one of the most extensively used has been the reaction of RNA with formaldehyde (Haselkorn & Doty, 1961; Penniston & Doty, 1963; Marciello & Zubay, 1964; Cox, 1969*a*). Since formaldehyde reacts with the primary amino groups of adenine, guanine and cytosine, which are hydrogen-bonded in the helical form (Fraenkel-Conrat, 1954; Staehelin, 1958), the rate and extent of reaction can distinguish hydrogen-bonded nucleotides from unbonded ones. The reaction of formaldehyde with free amino groups is more rapid than that with hydrogen-bonded amino groups. In other words, the formylation reaction can measure quantitatively the existence of hydrogen-bonded base-pairs in the RNA molecules, and their dissociation with increasing temperature (Boedtker, 1967; Cox, 1969*a*). The formylation reaction is conveniently followed spectrophotometrically since it is accompanied by an increase in the absorption in the 255–285nm region as well as a shift of the absorption maximum to a higher wavelength of 270nm. The results of the formylation reaction with microsomal RNA have been found by Hall &

Doty (1959) to be consistent with conclusions arrived at by other procedures with respect to hydrogen-bonded structure of the same RNA. Recently, similar observations were made by Cox (1969*a*,*b*) with reticulocyte rRNA.

In the present investigation slices of goat brain-cortex tissues were treated with mescaline. From the washed slices ribosomes were isolated and purified. RNA species were extracted from these ribosomes. The degree of hydrogen-bonded structure in the extracted RNA species was estimated on the basis of thermal and formylation reaction hyperchromicities, and the resultant loss of hydrogen-bonding in these constituent RNA species due to mescaline-treatment of brain-cortex slices was evaluated. Evidence is presented in this paper that mescaline affects the hydrogen-bonded structure of rRNA species of brain-cortex slices.

METHODS AND MATERIALS

Treatment of brain-cortex slices with mescaline. The treatment was the same as described in the preceding paper (Datta & Ghosh, 1970); the dose of mescaline was 0.01 mg/g of brain-cortex tissue slices.

Preparation of ribosomes. The ribosomes were prepared from the untreated and drug-treated slices and their purity was judged by the method of Datta & Ghosh (1963*b*).

Extraction of RNA. rRNA was extracted by the method of Kirby (1956) as described by Littauer & Eisenberg (1959). The precipitated RNA was dissolved in water and further purified by passing through a column (2 cm × 30 cm) of Sephadex G-25 that had been equilibrated with water as described by Martin (1966).

Sucrose-density-gradient centrifugation. Centrifugation was carried out in a Spinco Model L ultracentrifuge with an SW 25 head according to the procedure described by Martin (1966). Sucrose density gradients were prepared from 12.5 ml each of 25 and 5% (w/v) sucrose solutions (previously sterilized by autoclaving) in 0.1 M-sodium acetate buffer, pH 5.0. The 3 ml portions of rRNA were layered on to the gradients and centrifugation was carried out at 2°C for 15 h at 22500 rev./min. At the end of this period 1 ml fractions were collected from the bottom of the tubes and E_{260} values were measured. Sedimentation values were calculated from the relative position in sucrose density gradients by the Martin & Ames (1961) approximation procedure.

Chemical determinations. Each RNA preparation was analysed for phosphorus, nitrogen, DNA, protein, lipids, hexoses and hexosamine and the nucleotide composition. Most of the chemical determinations were carried out by methods indicated in the preceding paper (Datta & Ghosh, 1970). Total lipids were extracted and determined by the method of Folch, Lees & Sloane Stanley (1957). Hexoses and hexosamines were determined by the methods of Hess & Slade (1956) and of Elson & Morgan (1933) respectively.

Enzyme assays. Ribonuclease, phosphomonoesterase and phosphodiesterase activities were measured by methods indicated in the preceding paper (Datta & Ghosh, 1970).

Alkaline hyperchromicity. Ribosomes and extracted rRNA species were hydrolysed with 0.3 M-KOH for 18 h at 37°C. The hydrolysates were freed from protein (in the case of ribosomes) by treatment with M-$HClO_4$ and, after neutralization with KOH, E_{260} values were measured.

Determination of thermal hyperchromicity. The extracted rRNA species (about 20–25 μg/ml) were dissolved in 28 mM-acetate–veronal buffer, pH 7.0, containing 0.116 M-NaCl (Michaelis, 1931), and heated for 20 min at various temperatures in a thermostatic heating chamber (sensitivity ±1°C) attached to a Beckman DU Spectrophotometer in stoppered 1 cm cuvettes. The E_{260} values were measured over a range of temperatures between 30°C (room temperature) and 85°C during heating and also during cooling. The percentage increase of E_{260} or thermal hyperchromicity between 30 and 85°C was calculated on the basis of the E_{260} at 30°C. The thermal hyperchromicity values plotted in Figs. 2, 3 and 4 were averages of the values (which did not vary by more than 0.5%) obtained during heating and subsequent slow cooling of the rRNA solutions in the thermostatic heating chamber, with proper corrections being made for changes in concentrations of rRNA as a result of thermal expansion of water. The temperatures recorded were those of the rRNA solutions in the cuvettes.

Determination of formaldehyde hyperchromicity. The extracted rRNA species (about 20–25 μg/ml) were dissolved in 28 mM-acetate–veronal buffer, pH 7.0 containing 2% (w/v) of formaldehyde (G.R. quality of E. Merck A-G., Darmstadt, Germany) and the solutions were kept at 30°C for 6 h. The corresponding blanks were run. At definite time-intervals the E_{270} values of the solutions were measured in a Beckman DU Spectrophotometer in stoppered 1 cm cuvettes. The increase in extinction of the solutions ceased at about 6 h. Next the solutions were heated in the stoppered cuvettes in a thermostatic bath maintained at 70°C for 4 h. The E_{270} values of the solutions were read at time-intervals during the heating at 70°C. The percentage increase of E_{270} or formaldehyde hyperchromicity between 30 and 70°C was calculated on the basis of the extinction at 30°C in the absence of formaldehyde.

RESULTS

Studies on the purity of total rRNA preparations. The preparations of total rRNA species were checked for the presence of DNA, protein, hexoses and hexosamines, which could not be detected, within the assay limits, in the total rRNA preparations. The total rRNA preparations were found to be completely devoid of ribonuclease (acid and alkaline), phosphomonoesterase (alkaline) and phosphodiesterase activities, which appeared to be integral parts of the original ribosomes from which the rRNA species had been prepared by phenol extraction. The chemical compositions of total rRNA preparations presented in Table 1 do not show any significant difference in these respects between two types of total rRNA species isolated

Table 1. *Chemical composition, nucleotide composition and u.v.-extinctions of rRNA preparations*

Mean values ±S.E.M. of determinations with six different rRNA preparations are given.

	Untreated		Mescaline-treated	
Constituent	Total rRNA	28S rRNA	Total rRNA	28S rRNA
Nitrogen (mg/100mg)	14.0±0.5	13.9±0.5	14.2±0.6	14.0±0.6
Phosphorus (mg/100mg)	10.2±0.4	10.3±0.5	10.0±0.5	10.1±0.6
DNA, protein, lipids, hexoses and hexosamines	0	0	0	0
Diffusible 260nm-absorbing materials	0	0	0	0
Adenylate (mol/100mol)	22.8±1.0	22.5±1.1	22.9±1.1	22.6±1.2
Cytidylate (mol/100mol)	30.1±1.1	29.9±1.2	30.0±1.0	30.2±1.0
Guanylate (mol/100mol)	32.4±1.3	32.8±1.5	32.1±1.4	31.7±1.6
Uridylate (mol/100mol)	14.8±0.6	14.8±1.0	15.0±0.8	15.5±1.0
Ribonuclease, Phosphomonoesterase, Phosphodiesterase	0	0	0	0
$E_{260(P)}$ (in 14mM-acetate–veronal buffer, pH7.0)	8080±80	8030±100	8195±125	8170±90
$E_{260(P)}$ (in 0.1M-potassium phosphate buffer, pH7.0 plus 1mM-$MgCl_2$)	7590±65	7515±55	8090±60	8040±70
$E_{260(P)}$ (in 50mM-tris–HCl buffer, pH7.4, plus 4mM-$MgCl_2$ and 25mM-KCl)	7610±85	7585±60	8105±75	8125±90

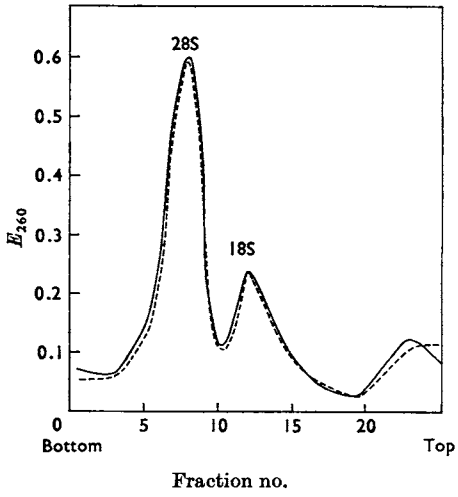

Fig. 1. Sucrose-density-gradient centrifugation of total rRNA preparation. The procedure and conditions were as described in the Methods and Materials section. ——, Total rRNA from untreated brain-cortex slices; ----, total rRNA from mescaline-treated brain-cortex slices.

in the present study. The u.v.-absorption spectra of total rRNA preparations show the characteristics of nucleic acids with absorption maxima at 259–260nm and absorption minima at 231–232nm. The E_{260}/E_{280} ratios of the untreated total rRNA and total rRNA from mescaline-treated preparations are both 1.90. The rRNA species do not differ significantly in their mean $E_{260(P)}$ values when measured in acetate–veronal buffer (Table 1). However, the total rRNA from mescaline-treated brains had slightly higher $E_{260(P)}$ values than those of total rRNA species of the untreated brains when the extinctions were measured in potassium phosphate buffer, pH7.0.

Sedimentation characteristics of total rRNA. The sucrose-gradient sedimentation analysis of total rRNA species isolated from the untreated brain-cortex slices showed two peaks with sedimentation coefficients of 28 and 18S respectively as calculated by the method of Martin & Ames (1961). Two similar peaks were observed when the total rRNA isolated from mescaline-treated brain-cortex slices was analysed by sucrose-density-gradient centrifugation under identical conditions (Fig. 1). Their sedimentation coefficients were also 28 and 18S. Our results are very close to those reported by Yamagami, Kawakita & Naka (1965), who found 28 and 17S components in rRNA species of guinea-pig brain-cortex, and by Hall & Doty (1959) who found 28 and 18S components in RNA prepared from microsomal particles of calf liver.

The three fractions (6, 7 and 8) with highest E_{260} values in the 28S peak were precipitated with

Table 2. *Hyperchromic values of ribosomes and their extracted RNA species*

Hydrolysis was with 0.3 M-KOH for 18 h at 37°C followed by removal of protein (in the case of ribosomes, with M-$HClO_4$) and neutralization (by dissolving in 0.1 M-potassium phosphate buffer, pH 7.0) or by digestion by pancreatic ribonuclease in 50 mM-tris–HCl buffer, pH 7.6, followed by removal of proteins and neutralization (by dissolving in 0.1 M-potassium phosphate buffer, pH 7.0). Mean values ±S.E.M. of determinations with five sets of ribosomes and rRNA preparations are given.

	Hyperchromicity (% increase in E_{260} after hydrolysis)					
	Untreated			Mescaline-treated		
Hydrolysis conditions	Ribosomes	Total rRNA	28S rRNA	Ribosomes	Total rRNA	28S rRNA
Alkaline	31.5±1.0	33.9±0.7	32.3±1.0	30.1±1.1	31.2±0.8	31.0±0.9
Ribonuclease	28.0±1.5	30.0±1.0	31.0±1.2	28.2±1.0	28.4±0.9	29.0±0.7

ethanol at −18°C. These fractions were next dissolved in water and passed through a short column (1 cm × 12 cm) of Sephadex G-25 for further purification. The 28S component was eluted as a single peak. The nucleotide compositions and u.v.-absorption spectra of the 28S rRNA component from the untreated and mescaline-treated brain-cortex slices were very similar and did not vary significantly from those of the corresponding total rRNA species (Table 1).

State of RNA within ribosomes and in extracted preparations. In an attempt to assess the state of RNA within the brain-cortex ribosomes the hyperchromic values of ribosomes, their extracted total RNA species and 28S rRNA species were determined either by alkaline hydrolysis or by pancreatic ribonuclease digestion. The percentage increases in E_{260} were determined after both hydrolysates had been freed of protein by treatment with ice-cold 2 M-perchloric acid and supernatants were brought to pH 7.0 in both alkaline and ribonuclease hydrolyses (similar treatments were made for ribosomes and extracted RNA species). The closely similar hyperchromic values (percentage increases in extinction) of ribosomes and the extracted total RNA species in ribosomes was more or less retained in the extracted RNA species, and the extraction procedure employed in the present study had a minimal effect on the configurational state of RNA species. From hydrolysis studies Schlessinger (1960) suggested that the configuration of extracted rRNA species (extracted by the phenol and detergent method of Kurland, 1960) in solution is almost the same as in the ribosomes of *Escherichia coli*. Recently, Cox (1969*a*) has observed that the conformation of the RNA moiety of reticulocyte ribosomes is essentially retained after removal of the protein. The hyperchromic effect of rat liver ribosomes has indicated that RNA bound with ribosomes, like free RNA, is partially hydrogen-bonded (Doty *et al.* 1959). It may be noted that the hyperchromic values due to hydrolysis of brain-cortex ribosomes and their extracted RNA species in the present study agree well with the values known for the RNA species from different sources such as yeasts, calf pancreas, pig liver and mouse liver (Magasanik, 1955).

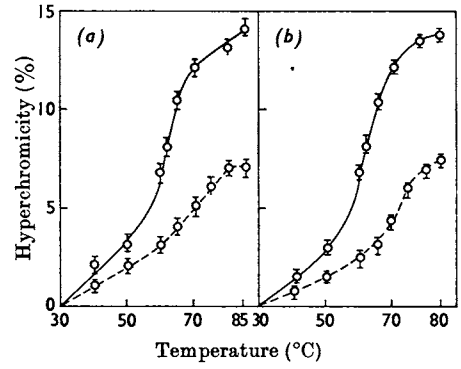

Fig. 2. Thermal hyperchromicity of (*a*) total rRNA and (*b*) 28S rRNA in 28 mM-acetate–veronal buffer, pH 7.0 containing 0.116 M-NaCl. The measurement and conditions were as described in the Methods and Materials section. ——, Untreated rRNA; – – – –, rRNA from mescaline-treated brain-cortex slices.

Studies of hydrogen-bonded structure of rRNA by thermal hyperchromicity. The percentage increase of E_{260} of the total rRNA solutions from the untreated and mescaline-treated brain-cortex slices as a function of temperature were noted during heating from 30 to 85°C as well as during slow cooling. As stated in the Methods and Materials section the E_{260} values at a particular temperature during heating and during slow cooling did not vary by more than 0.5% and the average values were used in the calculation of the percentage increases (Figs. 2, 3 and 4). The untreated brain-cortex total rRNA shows greater thermal hyperchromicity between 30 and 85°C in 28 mM-acetate–veronal

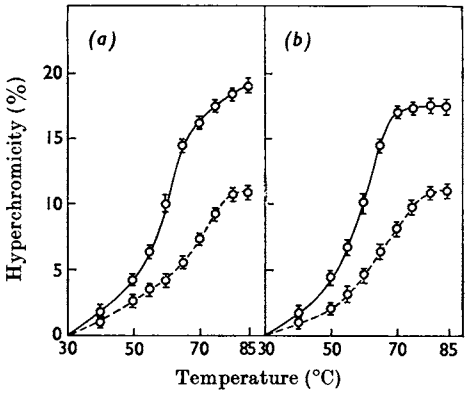

Fig. 3. Thermal hyperchromicity of (*a*) total rRNA and (*b*) 28S rRNA in 0.15M-potassium phosphate buffer, pH 7.0. The measurement and conditions are as described in the Methods and Materials section. ——, Untreated rRNA; – – – –, rRNA from mescaline-treated brain-cortex slices.

buffer, pH 7.0, containing 0.116M-NaCl (Michaelis, 1931) whereas the mescaline-treated brain-cortex total rRNA shows lower thermal hyperchromicity in this temperature range (Fig. 2*a*). From the curve it is evident that the 'melting temperature' (T_m) of the untreated brain-cortex total rRNA lies between 60 and 65°C. In the temperature range 30–85°C the increase of hyperchromicity of the mescaline-treated brain-cortex total rRNA is less distinct and T_m is not sharp. In other words, the T_m curve of the untreated brain-cortex total rRNA is comparatively steeper than that of the total rRNA from the mescaline treated brain-cortex slices. The decreased slope of the T_m region indicates that part of the hydrogen-bonded structure of this total rRNA has been lost. Similar observations were made with the 28S rRNA species of the untreated and mescaline-treated brain-cortex slices (Fig. 2*b*). That the rRNA species used during the thermal hyperchromicity measurements were of high molecular weight both before and after heating was known by (i) complete precipitability of the rRNA species by cold trichloroacetic acid or by perchloric acid without leaving any significant amount of acid-soluble nucleotides, (ii) identical sucrose- or caesium chloride-density-gradient sedimentation profiles, (iii) almost identical E_{260} values during heating and during slow cooling at a particular temperature and (iv) almost identical hyperchromic values on alkaline hydrolysis and pancreatic ribonuclease digestion. The different degrees of thermal hyperchromicity observed in the rRNA species of the untreated and mescaline-treated brain-cortex slices are not, therefore, apparently related to the degradation of rRNA species during heating.

The thermal stability of RNA in the transition from the double-helical to single-stranded forms depends on the electrolyte concentrations, and the temperature at which denaturation takes place increases when the concentration of electrolyte is increased within the range 1mM–1M (Dove & Davidson, 1962; Schildkraut & Lifson, 1965; Bellamy & Joklik, 1967; Cox & Kanagalingam, 1967; Tal, 1969). As presented above the thermal hyperchromicity values were determined in acetate–veronal buffer with a total ionic strength of 0.144M. It was decided to check whether these hyperchromic values were low owing to the low electrolyte concentrations of the buffer solutions in which rRNA species were heated or to the nature of the buffer used. Hence potassium phosphate buffers, equimolar and hypermolar to the acetate–veronal buffer used earlier, were used to determine the thermal hyperchromicity of the rRNA species (Figs. 3 and 4). In 0.15M-potassium phosphate buffer, pH 7.0 (Fig. 3) the thermal hyperchromicity values were higher than those obtained in acetate–veronal buffer (total ionic strength 0.144M) (Fig. 2) both for total rRNA species and 28S species of both the untreated and mescaline-treated brain-cortex slices. With more concentrated potassium phosphate buffer (0.25M, pH 7.0) the thermal hyperchromicity values increased further (Fig. 4). Although higher values of thermal hyperchromicity were obtained in phosphate buffer than in acetate–veronal buffer and at a greater ionic strength, the T_m values did not change very remarkably (Figs. 2, 3 and 4). The measurements of sedimentation profiles of the rRNA species used for determination of hyperchromicity values before and after heating to 85°C indicated that the rRNA species were not appreciably degraded during heating (Figs. 4*c*, 4*d*, 4*e* and 4*f*).

The greater thermal hyperchromic effect with the untreated brain-cortex total rRNA and 28S rRNA is indicative of a more highly organized hydrogen-bonded structure. On the basis of the assumption (which is approximately correct) that, on heating, the rRNA is reversibly disorganized, it is possible to estimate roughly the degree of organized hydrogen-bonded structure of the rRNA. As a result of the action of mescaline on brain-cortex slices the rRNA might have lost some of its hydrogen-bonded structure and on heating this rRNA would show a smaller hyperchromic effect. This smaller hyperchromic effect may be taken as an index of disorganization of the rRNA molecules. If the percentage of thermal hyperchromicity between 30 and 85°C for the untreated total rRNA be assumed as indicative of a 100% hydrogen-bonded structure, then the total rRNA from mescaline-treated slices

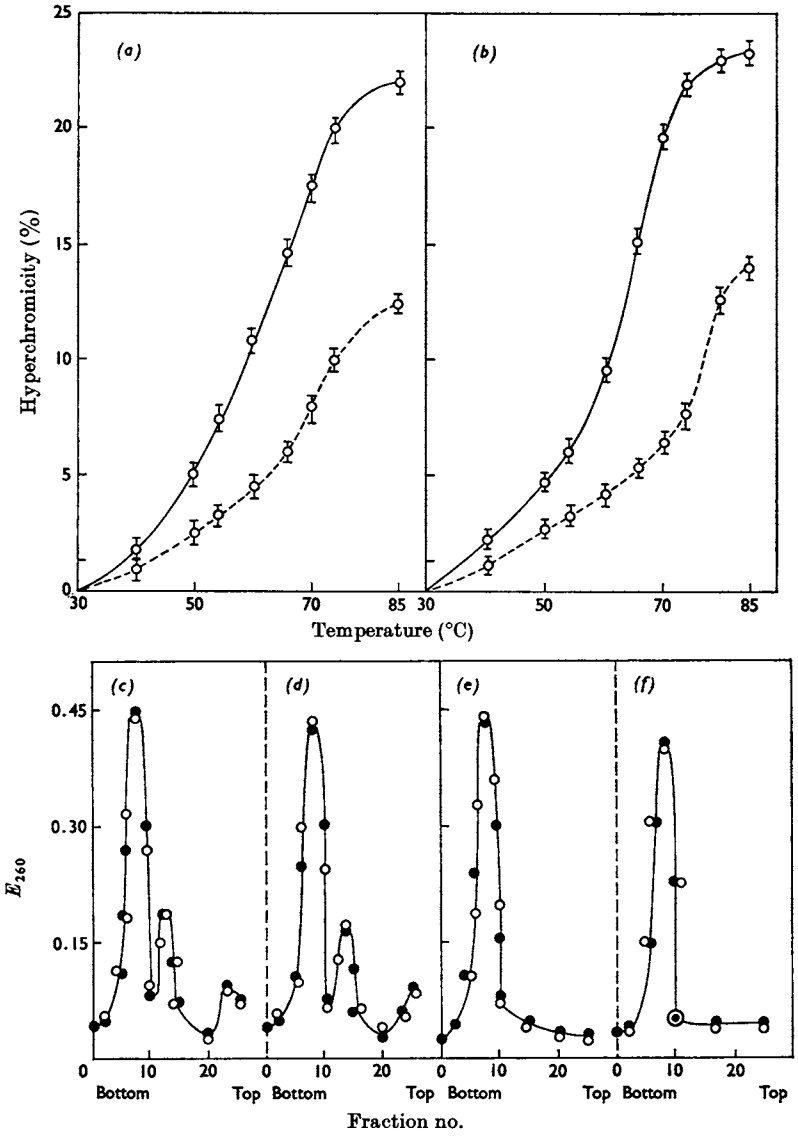

Fig. 4. (*a* and *b*) Thermal hyperchromicity of total rRNA (*a*) and 28 S rRNA (*b*) in 0.25 M-potassium phosphate buffer, pH 7.0. The measurement and conditions were as described in the Methods and Materials section. ——, Untreated rRNA; – – –, rRNA from mescaline-treated brain-cortex slices. (*c–f*) Sucrose-density-gradient centrifugation of total rRNA and 28S rRNA preparations before and after heating to 85°C. The procedure and conditions were as described in the Results and Methods and Materials sections. (*c*) Total rRNA from untreated brain-cortex slices: ●, before heating; ○, after heating. (*d*) Total rRNA from mescaline-treated brain-cortex slices: ●, before heating; ○, after heating. (*e*) 28S rRNA from untreated brain-cortex slices: ●, before heating; ○, after heating. (*f*) 28S rRNA from mescaline-treated brain-cortex slices: ●, before heating; ○, after heating.

has a 50% hydrogen-bonded structure (calculated from Fig. 2*a*). In other words, owing to the action of mescaline on brain-cortex slices the total rRNA of the drug-treated slices might have lost about 50% of its hydrogen-bonded structure. A calculation on the same basis suggests that the mescaline-treated brain-cortex 28S rRNA possesses only 54% of the hydrogen-bonded structure of the untreated

Table 3. *Thermal hyperchromicity and degree of hydrogen-bonded structure of rRNA species*

The basis of calculation was as described in the Results section. Results were taken from Figs. 2, 3 and 4.

Type of rRNA	% thermal hyperchromicity at 260nm between 30 and 85°C	% hydrogen-bonded structure (that of the untreated rRNA species being taken as 100)	% loss of hydrogen-bonded structure due to mescaline-treatment
Heated in 28mM-acetate–veronal buffer, pH7.0 (total ionic strength 0.144M) (Fig. 2)			
Untreated, total rRNA	14.0	100	50
Mescaline-treated, total rRNA	7.0	50	
Untreated, 28S rRNA	13.5	100	46
Mescaline-treated, 28S rRNA	7.3	54	
Heated in 0.15M-potassium phosphate buffer, pH7.0 (Fig. 3)			
Untreated, total rRNA	19.0	100	42
Mescaline-treated, total rRNA	11.0	58	
Untreated, 28S rRNA	17.5	100	37
Mescaline-treated, 28S rRNA	11.0	63	
Heated in 0.25M-potassium phosphate buffer, pH7.0 (Fig. 4)			
Untreated, total rRNA	22.0	100	43
Mescaline-treated, total rRNA	12.5	57	
Untreated, 28S rRNA	23.0	100	39
Mescaline-treated, 28S rRNA	14.0	61	

brain-cortex 28S rRNA (Fig. 2*b*). Similar calculations using the hyperchromic values obtained in 0.15M- and 0.25M-potassium phosphate buffer (Figs. 3 and 4) give the extent of loss of hydrogen-bonded structures of rRNA species of the mescaline-treated brain-cortex slices compared with the corresponding rRNA species of the untreated brain-cortex slices (Table 3). The mescaline treatment has caused a loss of 42–50% of the hydrogen-bonded structures in the total rRNA species and a loss of 39–46% of the hydrogen-bonded structures in the 28S rRNA species. Further experiments have indicated that mescaline has no direct effect on the hydrogen-bonded structure of the isolated normal total or 28S rRNA species when measured by thermal hyperchromicity in acetate–veronal or potassium phosphate buffer.

Studies on hydrogen-bonded structure of rRNA by formaldehyde hyperchromicity. The untreated brain-cortex total rRNA and total rRNA from mescaline-treated slices were treated for 6h with formaldehyde (final concentration 2%) in 28mM-acetate–veronal buffer (total ionic strength 0.144M) at 30°C. Next the solutions were quickly heated to and maintained at 70°C for 4h. In a preliminary study, it was observed that the formaldehyde reaction was accompanied by an increase in absorption in the 250–280nm region. The E_{270} values of the rRNA solutions were read at various time-intervals (Fig. 5).

Fig. 5*a* indicates that, in contrast with untreated brain-cortex total rRNA, that from mescaline-

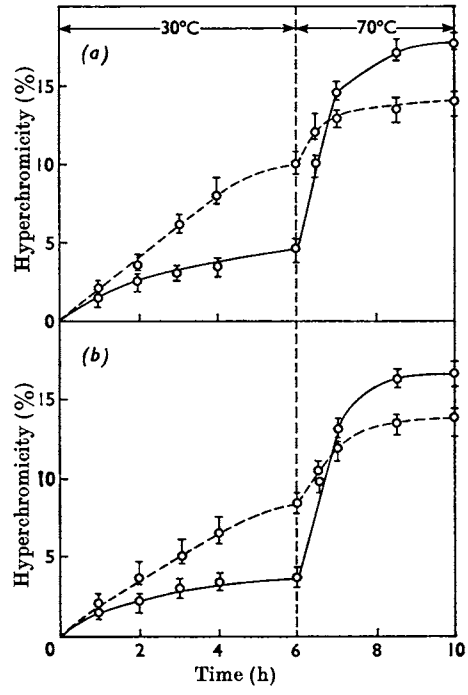

Fig. 5. Formaldehyde hyperchromicity of (*a*) total rRNA and (*b*) 28S rRNA in 28mM-acetate–veronal buffer, pH7.0, containing 0.116M-NaCl. The measurement and conditions were as described in the Methods and Materials section. ——, Untreated rRNA; – – – –, rRNA from mescaline-treated brain-cortex slices.

treated slices exhibits significantly greater formaldehyde hyperchromicity at 30°C, suggesting that the latter total rRNA has more non-hydrogen-bonded structure and more free amino groups, which react with formaldehyde at 30°C more rapidly to give rise to a greater hyperchromic effect at 270nm at this temperature. At a higher temperature (70°C) most of the hydrogen-bonding is lost and more amino groups of purine and pyrimidine bases are set free and hence the formylation reaction proceeds more rapidly and the hyperchromic effect becomes greater. At 70°C the untreated brain-cortex total rRNA exhibits a greater hyperchromic effect than the mescaline-treated brain-cortex total rRNA. Similar observations were made with the 28S rRNA species of the untreated and mescaline-treated brain-cortex slices (Fig. 5*b*).

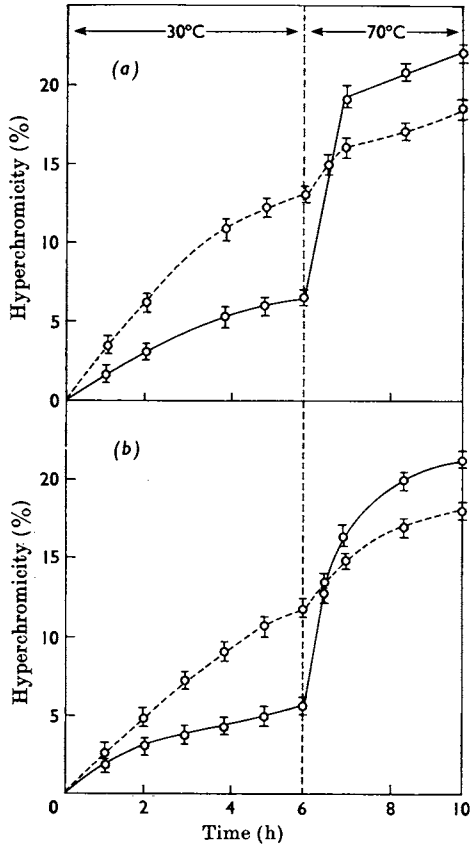

Fig. 6. Formaldehyde hyperchromicity of (*a*) total rRNA and (*b*) 28S rRNA in 0.15M-potassium phosphate buffer, pH7.0. The measurement and conditions were as described in the Methods and Materials section. ——, Untreated rRNA; – – – –, rRNA from mescaline-treated brain-cortex slices.

Since the heating of rRNA species in nearly equimolar potassium phosphate buffer gave greater thermal hyperchromicity values than in acetate–veronal buffer (Figs. 2 and 3 and Table 3), the formaldehyde hyperchromicity studies were also carried out in 0.15M-potassium phosphate buffer, pH7.0. As indicated in Fig. 6 and Table 4, the total rRNA and 28S rRNA species of both the untreated and mescaline-treated brain-cortex slices gave greater formaldehyde hyperchromicity values in 0.15M-potassium phosphate buffer than in acetate–veronal buffer. In potassium phosphate buffer the mescaline-treated brain-cortex total rRNA and 28S rRNA species also exhibited significantly greater formaldehyde hyperchromicity at 30°C than the respective untreated brain-cortex total rRNA and 28S rRNA. Similarly, in potassium phosphate buffer at 70°C, the untreated brain-cortex, total rRNA and 28S rRNA exhibited a greater hyperchromic effect than the corresponding mescaline-treated brain-cortex total rRNA and 28S rRNA.

The difference detailed above in formaldehyde hyperchromic effects of the two rRNA species indicates that the untreated brain-cortex total rRNA and 28S rRNA have more organized hydrogen-bonded structures than the respective preparations from mescaline-treated slices. Assuming that the formaldehyde hyperchromicity effect at 30°C represents the extent of non-hydrogen-bonded structure in rRNA, an approximate calculation is possible of the percentage of hydrogen-bonded structure in rRNA molecules. The above assumption provides, on calculation from Fig. 5*a*, the following percentage of hydrogen-bonded and non-hydrogen bonded structures: untreated brain-cortex total rRNA, 75% hydrogen-bonded structure (which permits formaldehyde reaction at 70°C) and 25% non-hydrogen-bonded structure (which permits formaldehyde reaction at 30°C); total rRNA from mescaline-treated slices, 28% hydrogen-bonded structure and 72% non-hydrogen-bonded structure (Table 4). Within the limits of the above assumption, it seems that the total rRNA of the mescaline-treated brain-cortex slices has lost 47% of its hydrogen-bonded structure compared with that of total rRNA of the untreated brain-cortex slices. Similar calculations were made for the total rRNA and 28S rRNA species from the formaldehyde hyperchromicity curves (Figs. 5 and 6) obtained by heating in acetate–veronal buffer and in potassium phosphate buffer and the results are shown in Table 4. It is evident that mescaline-treatment of brain cortex slices has caused a loss of 41–47% of the hydrogen-bonded structure in the total rRNA and a loss of 39–43% of the hydrogen-bonded structure in the 28S rRNA species.

Table 4. *Formaldehyde hyperchromicity and degree of hydrogen-bonded structure of rRNA species*

The basis of calculation was as described in the Results section. Results were taken from Figs. 5 and 6.

Type of rRNA	% formaldehyde hyperchromicity at 270nm: at 30°C after 6h	at 70°C after 4h more	% non-hydrogen-bonded structure	% hydrogen-bonded structure	% loss of hydrogen-bonded structure due to mescaline-treatment
Heated in 28mM-acetate–veronal buffer, pH7.0 (total ionic strength 0.144M) (Fig. 5)					
Untreated, total rRNA	4.5	17.8	25	75	47
Mescaline-treated, total rRNA	10.0	14.0	72	28	
Untreated, 28S rRNA	3.5	16.5	21	79	43
Mescaline-treated, 28S rRNA	8.5	13.4	64	36	
Heated in 0.15M-potassium phosphate buffer, pH7.0 (Fig. 6)					
Untreated, total rRNA	6.3	22.0	29	71	41
Mescaline-treated, total rRNA	13.0	18.5	70	30	
Untreated, 28S rRNA	5.5	21.3	26	74	39
Mescaline-treated, 28S rRNA	11.7	18.0	65	35	

Additional experiments by the formaldehyde hyperchromicity procedure have failed to show any direct effect of mescaline on the hydrogen-bonded structure of isolated normal total rRNA or 28S rRNA species.

DISCUSSION

Purification of rRNA by sucrose-density-gradient centrifugation followed by passage through a column of Sephadex G-25, and the absence of diffusible 260nm-absorbing materials (Table 1) preclude contamination of the total rRNA with small fragments of degraded RNA molecules. Since the total rRNA and 28S rRNA preparations are absolutely free from ribonuclease and other degrading enzymes (Table 1), the different extents of hydrogen-bonded structure observed in the rRNA preparations of the untreated and mescaline-treated brain-cortex slices are not in any way related to the degrading nucleases or degraded RNA molecules. That the rRNA species used during the thermal hyperchromicity measurements were of high molecular weight both before and after heating was known from the almost identical sucrose-density-gradient sedimentation profiles before and after heating (Figs. 4*c*, 4*d*, 4*e* and 4*f*). Almost identical E_{260} values of rRNA species during heating and during slow cooling at a particular temperature (Figs. 2, 3 and 4) suggest that the rRNA species are almost reversibly disorganized on heating and that these rRNA species remain almost undegraded during heating. The absence of any appreciable amounts of acid-soluble nucleotides after thermal hyperchromicity measurements also suggests that rRNA species are not detectably degraded into acid-soluble nucleotides and oligonucleotides during heating.

A comparison of the hyperchromic values in acetate–veronal buffer (Figs. 2 and 5) and those in equimolar potassium phosphate buffer (Figs. 3 and 6) suggests that the hyperchromicity of rRNA species depends on the nature of the buffer in which the RNA species are heated. The observed low hyperchromicity values of rRNA species in acetate–veronal buffer (Figs. 2 and 5) may be due to the buffer itself. According to Kyogoku, Lord & Rich (1968) barbiturates form strong specific hydrogen-bonding with adenine derivatives. In view of this observation it may be likely that when the rRNA species are heated in acetate–veronal buffer, the constituent barbital sodium, by forming hydrogen-bonds with adenine moieties, deters the disruption of hydrogen-bonds in rRNA molecules. Hence the hyperchromicity values are only partial in acetate–veronal buffer (Figs. 2 and 5). As stated above, higher hyperchromicity values are obtained when rRNA species are heated in equimolar or hypermolar potassium phosphate buffers (Figs. 3, 4 and 6).

In the present study, by using the thermal and formaldehyde hyperchromic effects to assess the hydrogen-bonded structures of rRNA species, evidence has been presented that rRNA of mescaline-treated brain-cortex slices possesses less hydrogen-bonded structure than rRNA of the untreated brain-cortex slices. Although the principles and assumptions on which the present two methods are based are quite arbitrary, the hyperchromic effects observed by the two methods

provided fairly consistent results about the loss of hydrogen-bonded structures of the mescaline-treated brain-cortex rRNA. However, in a number of studies where the hydrogen-bonded structures of RNA species are assayed by various kinds of measurements the results do not always agree quantitatively (Fresco, 1961; Ts'o, Helmkamp & Sander, 1961, 1962; Gould & Simpkins, 1969).

According to the studies of Schlessinger (1960), Bonhoeffer & Schchmann (1960), Cotter, McPhie & Gratzer (1967) and Cox (1969*a*) the extraction of RNA from ribosomes by the phenol method of Kirby (1956) (used in the present study) does not significantly decrease the hydrogen-bonded structure of the RNA moiety. Since the hyperchromicity values of brain-cortex ribosomes and their extracted RNA species as determined by hydrolysis by alkali and ribonuclease are fairly similar in the present study (Table 2) it is assumed that the state of hydrogen-bonding of RNA in ribosomes is more or less retained in the extracted RNA species. Comparison of the thermal hyperchromicity and formaldehyde reactivity of the two rRNA preparations from the untreated and mescaline-treated brain-cortex slices is possible, since these rRNA preparations have almost identical chemical and nucleotide compositions, u.v.-absorption and sedimentation characteristics. The differences observed in the thermal and formaldehyde hyperchromicity values of the two rRNA preparations are quite valid. Hence it may be inferred that the observed lowering of the degree of hydrogen-bonded structure in total rRNA of the mescaline-treated brain-cortex slices determined in the present study by thermal and formaldehyde hyperchromicity values (Figs. 2–6) might have resulted from the action of mescaline. This is also found to be true of the 28S rRNA component. In a previous study it was found that the convulsant drug strychnine and the central-nervous-system-stimulant drug nialamide {1-[2-(benzylcarbamoyl)ethyl]-2-isonicotinic acid hydrazide} also decreased the amounts of hydrogen-bonded structure of rRNA species of the drug-treated brain-cortex slices (Datta & Ghosh, 1964*a*). Since a direct effect of mescaline has been ruled out in the present study, the exact mechanism of how this reduction in the hydrogen-bonded structures of rRNA is brought about when mescaline acts on respiring brain-cortex tissue still remains a subject for further study.

Axelrod (1956) found that mescaline is demethylated by rabbit liver preparations. We have recently found that such demethylation of mescaline also takes place in brain-cortex slices and that rRNA, like other RNA species, is appreciably methylated (R. K. Datta, unpublished work). In view of this finding it is not unlikely that hydrogen-bonding sites in rRNA species are partially blocked by methylation on mescaline-treatment, leading to decreased hyperchromicity effects. In fact, Pillinger, Hay & Borek (1969) have observed that as methylation of tRNA increases, hyperchromicity correspondingly decreases.

Since the optical properties of the partly double-helical form appear to be the sum of the properties of hydrogen-bonded and non-hydrogen-bonded regions, the loss of hydrogen-bonded structure as observed in the present study should reflect itself in the higher $E_{260(P)}$ values of the rRNA species of the mescaline-treated brain-cortex slices. However, though $E_{260(P)}$ values reported for rRNA species of the mescaline-treated brain-cortex slices are slightly higher (6–7%) than those for rRNA species of the untreated brain-cortex slices (when measured in potassium phosphate buffer, pH 7.0, and tris–HCl buffer, pH 7.4) (Table 1), these higher values are not proportional to the extent of loss of hydrogen-bonding reported in Figs. 2–6. This discrepancy is difficult to explain directly. According to Borek & Christman (1965) a minimum of 5% decrease in E_{260} values occurs on methylation of tRNA by homologous RNA methylase. Ludlum, Warner & Wahba (1964) previously found a decrease in E_{260} values of the same order of magnitude on chemical methylation of polyadenylic acid. As methylation of rRNA occurs in mescaline-treated brain-cortex slices (R. K. Datta, unpublished work), the so-methylated rRNA species should have lower $E_{260(P)}$ values than those of the untreated brain-cortex slices. On the other hand, as the hydrogen-bonds are lost on mescaline-treatment the $E_{260(P)}$ values should increase. These two opposing processes i.e. methylation leading to decreased $E_{260(P)}$ and loss of hydrogen-bonds leading to increased $E_{260(P)}$ may account for a slight change in $E_{260(P)}$ values (when measured in potassium phosphate buffer, pH 7.0, and tris–HCl buffer, pH 7.4) of mescaline-treated brain-cortex rRNA species compared with the untreated brain-cortex rRNA species (Table 1).

The thermal hyperchromicity values for rRNA species of brain-cortex tissue (up to 23% increase in E_{260} over 30 to 85°C) as reported in the present study (Figs. 2–4) appear to be somewhat low in comparison with those for rRNA species of liver tissue (Magasanik, 1955; Doty *et al.* 1959) or of reticulocytes (Cox & Kanagalingam, 1967) (about 25–35% increase in E_{260} over 25 to 95°C). This discrepancy cannot be fairly explained owing to lack of adequate information about the secondary structures of brain ribosomes and rRNA (reviewed by Datta, 1966). It is believed that the thermal hyperchromicity of the ordered secondary structure of RNA increases as the number of guanine–cytosine base-pairs increases (Dove & Davidson, 1962; Schildkraut & Lifson, 1965; Bellamy &

Joklik, 1967). Although our preparations of brain-cortex total rRNA and 28S rRNA have almost the same proportions of guanine and cytosine base residues as liver rRNA species (Magasanik, 1955; Chauveau, Moule, Rouillier & Schneebeli, 1962; Goswami, Barr & Munro, 1962), nothing is known regarding the proportions of these base residues that form base-pairs in brain rRNA species giving rise to the ordered secondary structure of brain rRNA. According to some estimates (Doty, Boedtker, Fresco, Haselkorn & Litt, 1959; Spirin, 1963; Cox, 1966), about 50–70% of these base residues form base-pairs. The average length of the 'hairpin loop' has been estimated to be 10–20 residues and an upper limit of 35 residues has been established for reticulocyte rRNA species (Cox & Kanagalingam, 1967). Moreover, there appears to be an appreciable dependence of hyperchromicity on helix length of short helices (Rich & Tinoco, 1960) and the helix content of reticulocyte rRNA species as determined by different methods varies between 50 and 80% (Gould & Simpkins, 1969). These apparently broad limits with respect to ordered secondary structure *vis-à-vis* hyperchromicity values allow no satisfactory explanation regarding the lower hyperchromicity values of rRNA species from brain-cortex tissues. Detailed studies are desirable to obtain adequate information in this area so that a quantitative comparison may be made of the hyperchromic values of rRNA species isolated from brain and from liver and other tissues.

Selective hydrogen-bond formation is an important component of biological organization. This is especially true in nucleic acids, which are said to constitute the principal cerebral components of learning and behaviour. Although there is no known relevance of the observed lowering of hydrogen-bonded structures of RNA to the pharmacological activities of mescaline, the previous observations that convulsant, central-nervous-system-stimulant and psychotomimetic drugs affect the stability of ribosomal particles (Datta & Ghosh, 1963*a*, 1964*b*, 1965, 1970; Ghosh, *et al.* 1965; Ghosh, Datta, Chanda & Sikdar, 1962), together with the present observations, appear to point towards a relationship between the neurotropic effects of these drugs and the structural integrity of the RNA molecules of brain cells.

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