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Mescaline-Induced Changes of Brain-Cortex Ribosomes

EFFECT OF MESCALINE ON THE STABILITY OF BRAIN-CORTEX RIBOSOMES

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1. During the action of mescaline sulphate on goat brain-cortex slices the ribosomal particles become susceptible to breakdown, releasing protein, RNA, acid-soluble nucleotides and ninhydrin-positive materials, resulting in loss of ribosomal enzyme activities. 2. Ribosomes of the mescaline-treated cortex slices undergo rapid degradation in the presence of trypsin and ribonuclease. 3. Mescaline does not alter the chemical and nucleotide compositions or the u.v.-absorption characteristics of ribosomal particles, however.

Previous studies have demonstrated that various convulsant drugs effect significant histological and biochemical changes in the ribonucleoprotein constituents of nerve tissue (Ghosh, Datta, Chanda & Sikdar, 1962). When brain-cortex slices are treated with central-nervous-system-stimulant drugs, e.g. isoniazid (isonicotinic acid hydrazide) and nialamide {1-[2-(benzylcarbamoyl)ethyl]-2-isonicotinic acid hydrazide}, the ribosomal particles become more susceptible to breakdown, releasing protein, RNA and acid-soluble nucleotides (Datta & Ghosh, 1963*a*). Similar changes in the stability of ribosomes occur during the action of convulsant drugs, e.g. strychnine and picrotoxin (Datta & Ghosh, 1965). A single intraperitoneal or intravenous dose of 5–15mg/kg body weight of mescaline sulphate (3,5,6-trimethoxyphenethylamine sulphate) produces hyper-reflexia of limbs and static tremors in rats and rabbits and apparently impairs their visual and space perception (Ghosh *et al.* 1962). In the present study the action of mescaline sulphate on the ribosomal particles of goat brain-cortex tissue was investigated. The use of goat brain in this study was prompted by its larger size and easy availability from slaughterhouses. The results indicate that the stability of ribosomal particles is affected as a result of mescaline treatment.

METHODS AND MATERIALS

Treatment of brain-cortex slices with mescaline. The cortex portions of brain tissue obtained from freshly slaughtered goats were carefully removed and cut into 0.5mm slices in the cold with a Stadie–Riggs slicer. Approx. 10g wet wt. of cortex slices was suspended in 100ml of Krebs–Ringer buffer, pH7.0, containing 0.5M-glucose, that had previously been saturated with O_2+CO_2 (95:5). The respiring conditions of the slices were periodically checked by separate manometric experiments carried out under similar conditions. After incubation at 37°C for 2h the slices were collected by centrifugation and washed four times with 100ml portions of Krebs–Ringer buffer. Unless otherwise stated, 'mescaline-treated' cortex slices indicate glucose-plus-mescaline-treated slices whereas 'untreated' (normal) slices mean slices treated with glucose alone.

Preparation of ribosomes. Ribosomes were prepared from the untreated and mescaline-treated brain-cortex slices by using deoxycholate as described by Datta & Ghosh (1963*b*). Ribosomes were further purified by layering over a discontinuous sucrose density gradient consisting of 9ml of 2M-sucrose and 11ml of 1M-sucrose (previously sterilized by autoclaving) and by centrifuging for 15h at $60000g_{av.}$ in a Spinco SW25 rotor to collect a pellet of free ribosomes. The purity of the ribosomal preparation was judged as described by Datta & Ghosh (1963*b*). The sedimentation pattern of these ribosomes showed a main peak with a sedimentation coefficient (in 0.05M-tris–HCl buffer, pH7.6 plus mM-$MgCl_2$) of 80S together with four minor components, 15S, 50S, 70S and 120S, the concentration of each component being approx. 65, 7, 10, 10 and 8% respectively (Ghosh *et al.* 1962). When assayed by the procedure of Acs, Neidle & Waelsch (1961) the ribosomes of brain-cortex tissue were quite active in incorporating leucine (1.2–1.5nmol/mg of ribosomal protein per 30min) into acid-insoluble protein in a cell free system (R. K. Datta, unpublished work).

Chemical determinations. Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951) with crystalline bovine serum albumin as standard. Nitrogen was determined by the modified micro-Kjeldahl method described by Ma & Zuazaga (1942). Organic phosphorus compounds were digested by the method of King (1932) and inorganic phosphorus was determined by the method of Lowry, Roberts, Wu, Hixon & Crawford (1954). RNA was determined by the orcinol method of Mejbaum (1939) and also by the u.v.-spectrophotometric

method of Hotchkiss (1957). Nucleotides obtained after hydrolysis with 0.3 M-KOH at 37°C for 18 h were separated by paper chromatography by the method of Magasanik, Vischer, Doniger, Elson & Chargaff (1950) and the amounts of the respective nucleotides were calculated by using the factors given by Elson, Gustafson & Chargaff (1954). The concentration of acid-soluble nucleotides was determined spectrophotometrically assuming an E_{260} value of 33 for a solution containing a nucleotide mixture obtained from complete hydrolysis of 1 mg of yeast RNA/ml in 5 mM-magnesium cacodylate buffer, pH 7.0. Amino acids were determined (as glutamic acid equivalents) by the ninhydrin method of Moore & Stein (1948).

Enzymic assays. Ribonuclease activity was measured by the method of McDonall (1955) on the basis of the liberation of acid-soluble nucleotides from the purified, highly polymerized and well-dialysed samples of yeast RNA used as the substrate. The reaction was stopped by adding an equal volume of ice-cold uranyl acetate–trichloroacetic acid solution (0.25% uranyl acetate in 2.5% trichloroacetic acid). The clear supernatant was collected by centrifugation and the liberated acid-soluble nucleotides were measured by the u.v.-spectrophotometric method of Hotchkiss (1957) and checked occasionally by the orcinol method of Mejbaum (1939). The details of the assay have been described elsewhere by Datta, Bhattacharyya & Ghosh (1964). The specific activity is expressed as μg of yeast RNA hydrolysed/mg of ribosomal protein per h.

Phosphomonoesterase activity was measured by the method of Bessey, Lowry & Brock (1946) with *p*-nitrophenyl phosphate as the substrate. The incubation was stopped by adding 4 vol. of cold 0.25 M-NaOH to give a final NaOH concentration of 0.20 M. The clear supernatant was collected by centrifugation and its E_{400} value was measured. The amounts of *p*-nitrophenol liberated during the incubation were known from a calibration curve drawn with known amounts of *p*-nitrophenol dissolved in 0.20 M-NaOH. The specific activity of phosphomonoesterase is expressed as nmol of *p*-nitrophenol liberated/mg of ribosomal protein per h. This procedure was described in detail by Datta & Ghosh (1964).

Phosphodiesterase activity was measured by the method of Frisch-Niggemeyer & Reddi (1957) with bis-*p*-nitrophenyl phosphate as the substrate. The procedure, which was basically the same as that for phosphomonoesterase, has been described in detail by Datta & Ghosh (1963c) and Datta, Ghosh & Bhattacharyya (1969). The specific activity of the phosphodiesterase is expressed as nmol of *p*-nitrophenol liberated/mg of ribosomal protein per h. Occasionally, the activity was checked by using adenosine 2′:3′-cyclic phosphate as a substrate as described by Drumond, Iyer & Keith (1962). Trypsin digestion was carried out by the method of Anson (1938) as described by Marks, Datta & Lajtha (1968).

Enzymes. Crystalline pancreatic ribonuclease and twice-recrystallized trypsin were purchased from Sigma Chemical Co., St Louis, Mo., U.S.A. Trypsin was checked for the absence of ribonuclease activity by the procedure of Piatigorsky (1968).

Drug. Synthetic crystalline mescaline sulphate (prism-shaped, m.p. 184°C) was obtained from Hoffman–LaRoche and Co. Ltd., Basle, Switzerland. The purity of the drug was over 99.5% based on the estimation of carbon (Found, 62.0%; expected, 62.2%) and nitrogen (Found, 6.60%; expected, 6.63%). Paper chromatography with butanol–acetic acid–water (4:1:5, by vol.) (Daly, Axelrod & Witkop, 1962) followed by staining with Gibb's reagent (Senoh, Daly, Axelrod & Witkop, 1959) gave a single spot, indicating the absence of any congener of mescaline. The presence of high-molecular-weight substances in the drug could not be detected by trichloroacetic acid or perchloric acid. The drug was free from ribonuclease contamination since when incubated in the presence of highly polymerized and well-dialysed samples of yeast RNA the drug liberated neither acid-soluble nucleotides absorbing at 260–280 nm nor orcinol-positive acid-soluble materials. The drug was also free from any phosphomonoesterase, phosphodiesterase and trypsin contamination. The drug was neutralized before use.

Buffers. 28 mM-Acetate–veronal buffer, with pH ranging from 2.5 to 9.6 was prepared as described by Michaelis (1931). 5 mM-Magnesium cacodylate buffer was prepared as described by Gillchriest & Bock (1958), who reported that this buffer gave improved yield and excellent stability of the 80S class of ribonucleoproteins from various sources. Other buffers were prepared with the compositions given by Dawson, Elliott, Elliott & Jones (1959). Krebs–Ringer buffer was prepared as described by Umbreit, Burris & Stauffer (1957).

RESULTS

Chemical and nucleotide compositions of ribosomes of mescaline-treated brain-cortex slices. Table 1 shows the chemical and nucleotide compositions of ribosomal particles isolated from the mescaline-treated brain-cortex slices. These results indicate that mescaline treatment of brain-cortex slices at a pharmacological concentration of 0.01 mg of drug/g wet wt. of tissue (Smythies, Bradley & Johnston, 1967) causes no significant changes in the chemical and nucleotide compositions of ribosomes. The u.v.-absorption spectra of ribosomes of the mescaline-treated slices, under similar conditions, reveal no significant difference from the u.v.-absorption spectra of ribosomes isolated from the untreated cortex slices.

Enzymic activities of ribosomes of mescaline-treated brain-cortex slices. Goat brain-cortex ribosomes possess acid ribonuclease, alkaline phosphomonoesterase and alkaline phosphodiesterase (Datta *et al.* 1964; Datta & Ghosh, 1963c, 1964; Datta *et al.* 1969). These enzymes appear to be integral parts of brain-cortex ribosomes, since they cannot be removed or separated by further purification and manipulation of ribosomes. Attempts to separate these enzymes from ribosomes by sucrose- and caesium chloride-density-gradient centrifugations, by starch-gel electrophoresis, by treatment with sodium deoxycholate or sodium dodecyl sulphate or by treatment with salts or urea have been unsuccessful. Studies by Pirie (1957), Elson (1959), Spahr & Hollingworth (1961), Kihara, Hu &

Table 1. *Chemical and nucleotide compositions of ribosomes of untreated and mescaline-treated brain-cortex slices*

Treatment of brain-cortex slices with mescaline and preparation of ribosomes were as described in the Methods and Materials section. Mean values ±S.E.M. of determinations with six different ribosomal preparations are given.

Constituent	Ribosomes isolated from brain-cortex slices previously treated with: Glucose (0.5 M)	Glucose (0.5 M)+mescaline (0.01 mg/g wet wt. of brain-cortex slices)
Nitrogen (mg/100 mg)	14.7±0.9	14.2±1.1
Phosphorus (mg/100 mg)	3.4±0.2	3.3±0.3
Protein (mg/100 mg)	70.0±0.2	71.0±1.5
RNA (mg/100 mg)	30.0±1.0	29.0±1.2
Adenylate (mol/100 mol)	22.6±0.9	22.9±1.1
Cytidylate (mol/100 mol)	30.4±1.1	30.0±1.5
Guanylate (mol/100 mol)	32.6±1.2	32.1±1.1
Uridylate (mol/100 mol)	14.4±0.6	15.0±0.8
U.v.-absorption		
Maximum (nm)	260	260
Minimum (nm)	230	230
E_{260}/E_{280} ratio	1.70	1.70
$E_{260(P)}$ (in 14 mM-acetate–veronal buffer, pH 7.0)	7420–7540	7340–7450
$E_{260(P)}$ (in 50 mM-tris–HCl buffer, pH 7.4, plus 4 mM-$MgCl_2$ and 25 mM-KCl)	7500–7595	7690–7725
$E_{260(P)}$ (in 0.1 M-potassium phosphate buffer, pH 7.0, plus 1 mM-$MgCl_2$)	7535–7605	7675–7755

Halvorson (1961*b*), De Lamirande, Boileau & Rorais (1966) and Ursino & Alberghina (1968) have indicated that ribonuclease, deoxyribonuclease and β-glucosidase found in association with ribosomes are integral parts of ribosomes of various bacterial, plant and animal sources. The present experiments were undertaken to check whether mescaline-treatment of brain-cortex slices has any effect on ribosomal ribonuclease, phosphomonoesterase and phosphodiesterase activities.

The results in Table 2 demonstrate some general decrease in the specific activities of the above-named enzymes in the ribosomes isolated from the untreated slices. This decrease of specific activities of the enzymes is time-dependent for up to 5 h of drug treatment. Since these enzymes are apparently integral parts of brain-cortex ribosomes, the observed decreases of their specific activities reflect some alteration in the ribosomes themselves.

Effect of mescaline on the enzymic activitites of ribosomes of fresh brain-cortex tissue. In view of the general decrease in the specific activities of the ribosomal enzymes of mescaline-treated brain-cortex slices (Table 2) it seemed important to check whether this inactivation of ribosomal enzymes is due to the direct effect of mescaline on ribosomes. This was checked by measuring enzymic activities of fresh ribosomes (isolated from normal brain-cortex tissue) in the presence of mescaline. The percentage activities of ribonuclease, phosphomonoesterase and phosphodiesterase in the presence of a relatively high dose of mescaline (1 μg/mg of ribosomal protein) are shown in Table 3. These results indicate that when ribosomes of fresh brain-cortex tissue are incubated *in vitro* with mescaline no significant inhibition of the enzymic activities occurs.

Stability of ribosomes of mescaline-treated brain-cortex slices. A comparison of the percentage loss of specific activities of ribosomes of mescaline-treated slices (Table 2) with the percentage loss of specific activities of ribosomes isolated from normal brain cortex and later incubated in the presence of mescaline (Table 3) leads to a difficult situation, i.e. mescaline appears to have no primary effect on enzymic activity when it acts *in vitro* on the isolated ribosomes whereas it appears to have a significant inhibitory effect on the enzymes of ribosomes of brain-cortex slices treated with the drug. It appears that the lower specific activities of enzymes in the former case (Table 2) may not be due to the direct effect of mescaline on ribosomes. It is not unlikely that mescaline has acted on the ribosomal particles in an indirect manner, i.e. by altering environmental conditions, such as pH, ionic strengths etc. surrounding the ribosomes in the tissue. Mescaline

Table 2. *Relative specific activities of ribosomal enzymes isolated from untreated and mescaline-treated brain-cortex slices*

Treatment of brain-cortex slices with mescaline and the preparation of ribosomes were as described in the Methods and Materials section. The isolated ribosomes were assayed for enzymic activities at their respective optimum pH values. The ribonuclease assay systems contained 0.5mg of ribosomal protein, 2.5mg of purified and dialysed yeast RNA and 14mM-acetate–veronal buffer, pH5.4, in a total volume of 1ml. Other details were as described by Datta *et al.* (1964) and in the Methods and Materials section. The phosphomonoesterase assay systems contained 0.5mg of ribosomal protein, 5mM-*p*-nitrophenyl phosphate and 14mM-acetate–veronal buffer, pH8.1, in a total volume of 2ml. Other details were as described by Datta & Ghosh (1964) and in the Methods and Materials section. The phosphodiesterase assay systems contained 0.5mg of ribosomal protein, 5mM-bis-*p*-nitrophenyl phosphate and 14mM-acetate–veronal buffer, pH8.9, in a total volume of 2ml. Other details were as described by Datta & Ghosh (1963*c*) and Datta *et al.* (1969) and in the Methods and Materials section. The specific activities of these enzymes were defined in the Methods and Materials section. Mean values ±S.E.M. of five different experiments are given. Values in parentheses indicate the mean specific activities of the respective enzymes.

Ribosomes isolated from brain-cortex slices previously treated with	Relative specific activities (%)		
	Ribonuclease	Phosphomonoesterase	Phosphodiesterase
Glucose (0.5M)	100±0 (170)	100±0 (400)	100±0 (50)
Mescaline (0.01mg/g wet wt. of brain-cortex slices)	66±3	52±2	47±3
Glucose+mescaline	87±2	72±3	70±1

Table 3. *Effect of mescaline on enzymic activities* in vitro *of ribosomes of fresh brain-cortex tissue*

Ribosomes were isolated from fresh brain-cortex tissue as described in the Methods and Materials section. The enzymic activities were assayed in the presence of mescaline (1μg/mg of ribosomal protein) at their respective optimum pH values. The assay system and other details were as described in Table 2. Figures in parentheses indicate the mean specific activities of the respective enzymes as defined in the Methods and Materials section. Mean values ±S.E.M. of five different experiments are given.

	Activity (%)		
	Ribonuclease	Phosphomonoesterase	Phosphodiesterase
Glucose (0.5M)	100±0 (174)	100±0 (408)	100±0 (48)
Glucose (0.5M)+mescaline (0.01mg/g wet wt. of brain-cortex slices)	99±2	98±1	96±2

might have initiated some instability in the macromolecular structure of ribosomes. If so, the induced instability of ribosomes isolated from mescaline-treated slices may account for the decrease in specific activities of ribosomal enzymes. This has been found to be true during the action of other psychotropic drugs (Datta & Ghosh, 1963*a*, 1965; Ghosh, Datta & Bhattacharyya, 1965). Hence an experiment was carried out to examine the degree of instability that may have been induced in ribosomal particles during the action of mescaline.

It is well known that the ribosomal particles are susceptible to degradation in the presence of high sodium chloride or potassium chloride concentrations (McQuillen, 1961) or to the absence of Mg^{2+} ions (Shigera & Chargaff, 1960; Ts'o & Vinograd, 1961). It is likely that some changes in the concentration of these ions may occur during the action of mescaline. The decreased stability of biomacromolecules is usually studied by measuring the release of low-molecular-weight components therefrom. Kihara, Halvorson & Bock (1961*a*) and Eardman, Thomas, Norton & Herbst (1968) studied the stability of ribosomal particles on the basis of release of various components in some selected media. In the present study also the effect of mescaline treatment on the stability of ribosomal particles was tested by measuring the rate of degradation of ribosomes into their components in the various suspension media that are often selected for such degradation studies.

The choice of different suspension media was made on the basis of observations that the stability of ribosomal particles is affected by high salt concentrations (Shigera & Chargaff, 1960), urea and phosphate (Kihara *et al.* 1961*a*). In the

Table 4. *Release of components from ribosomes of untreated and mescaline-treated brain-cortex slices*

Ribosomes were isolated from untreated and mescaline-treated brain-cortex slices. Portions (20mg) of ribosomes containing 14mg of protein and 6mg of RNA were suspended in 5ml portions of 5mM-magnesium cacodylate buffer, pH7.0, in which the agents listed in the table were dissolved with pH adjusted where necessary and incubated at 37°C for 3h with controls. The suspensions were then spun at 105000$g_{av.}$ for 1h and the supernatants were carefully decanted and collected. Protein and RNA were determined in the 5%-trichloroacetic acid-precipitated residues, and the acid-soluble nucleotides and amino acids were determined in the trichloroacetic acid supernatants. Mean values of determinations with six different ribosomal preparations are given.

Ribosomes isolated from brain-cortex slices previously treated with	Component released	Amount of component (mg/5ml) released in suspension medium of: Control (5mM-magnesium cacodylate buffer, pH7.0)	NaCl (0.5M)	Phosphate (0.5M)	Urea (4M)	EDTA (0.1M)	Spermidine (5mM)
Glucose (0.5M)	Protein	0.20	1.35	1.06	2.08	0.40	0.14
	RNA	0.09	0.32	0.24	0.35	0.40	0.04
	Acid-soluble nucleotides	0.016	0.144	0.072	0.160	0.172	0.011
	Amino acids	0.022	0.101	0.120	0.147	0.032	—
Glucose (0.5M) +mescaline (0.01mg/g wet wt. of brain-cortex slices)	Protein	0.66	2.14	1.58	3.82	0.87	0.40
	RNA	0.31	0.60	0.49	0.65	0.67	0.21
	Acid-soluble nucleotides	0.114	0.234	0.234	0.336	0.354	0.080
	Amino acids	0.074	0.300	0.232	0.264	0.061	—

present study the relative stability of ribosomal particles from mescaline-treated slices was assayed in terms of the release of protein, RNA, acid-soluble nucleotides and ninhydrin-positive materials (mostly amino acids) in the suspension media containing these different substances. Since Gillchriest & Bock (1958) observed that ribonucleoprotein particles remained stable in 5mM-magnesium cacodylate buffer, pH7.0, magnesium cacodylate buffer of the same concentration and pH was used in the present study as a suitable control medium for the suspension of ribosomes. The release into different suspension media of protein, RNA, acid-soluble nucleotides and ninhydrin-positive materials from the ribosomes of the mescaline-treated brain-cortex slices is shown in Table 4. These results show that ribosomes isolated from mescaline-treated brain-cortex slices release more chemical constituents of ribosomes than do normal ribosomes. A general idea of the degree of stability of ribosomal particles may be gained from the release of protein, RNA, acid-soluble nucleotides and ninhydrin-positive materials from ribosomes on suspension in magnesium cacodylate buffer, or in the presence of sodium chloride, EDTA, urea and phosphate. In these suspension media there is release of more of the above-named materials from the ribosomes of mescaline-treated slices. It is well known that polyamines preserve the macromolecular structure and prevent the degradation of ribosomes (Eardman *et al.* 1968). Datta, Sen & Ghosh (1969) have observed that polyamines prevent the breakdown of brain-cortex ribosomes into smaller components. Hence spermidine was included in the present study to check whether the mescaline-induced instability of ribosomes of the drug-treated slices can be overcome in the presence of spermidine. When such ribosomes are incubated in the presence of spermidine (last column of Table 4) the release of the ribosomal components from the ribosomes of mescaline-treated slices is remarkably decreased in the spermidine-containing suspension medium compared with the release of these components in other media. This was also found to be true with spermine, putrescine and cadaverine. Although the release of components from ribosomes of mescaline-treated slices in polyamine-containing media is remarkably decreased the amounts of released components are quantitatively higher than those released from ribosomes of untreated brain-cortex slices. This again indicates that the macromolecular structure of ribosomes is affected by mescaline treatment and once it has been affected it cannot be completely reversed by polyamines, though its breakdown may be decreased to some extent by providing some polyamines in the medium.

Degradation of ribosomes by ribonuclease and trypsin. The susceptibility of ribosomes to enzymic degradation was determined by incubating ribosomes with pancreatic ribonuclease and trypsin and estimating the extent of degradation of RNA and

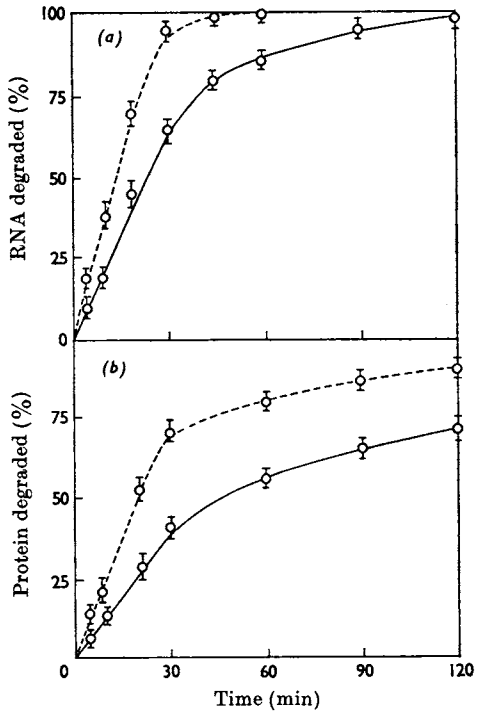

Fig. 1. (*a*) Digestion of brain-cortex ribosomes by pancreatic ribonuclease. The incubation system contained 10mg equivalent of ribosomal RNA, 1mg of crystalline pancreatic ribonuclease and 14mM-acetate–veronal buffer, pH7.5, in a total volume of 10ml. While incubation was carried out at 37°C samples were withdrawn and the rate of degradation of RNA was determined. (*b*) Digestion of brain-cortex ribosomes by trypsin. The incubation system contained 10mg equivalent of ribosomal protein, 1mg of crystalline trypsin and 14mM-acetate–veronal buffer, pH7.6, in a total volume of 10ml. While incubation was carried out at 37°C samples were withdrawn and the rate of degradation of protein was determined. ——, Untreated ribosomes; ----, mescaline-treated ribosomes.

protein constituents of ribosomes. The results are expressed as percentage of total RNA or protein present in the ribosomes degraded by the enzymes (Fig. 1). The ribosomes used in the study were isolated after 2h of drug treatment of brain-cortex slices. It is evident that ribosomes of the mescaline-treated cortex slices are more rapidly degraded by these enzymes than those of the untreated brain-cortex slices. It was also observed that the degree of differential degradation of ribosomes by ribonuclease and trypsin depends on the period of drug-treatment of brain-cortex slices before the isolation of ribosomes. This indicates that the ribosomes of mescaline-treated brain-cortex slices are more susceptible to enzymic degradation than the ribosomes of the untreated slices and that the mescaline effect is time-dependent.

DISCUSSION

Although various biochemical aspects of brain ribosomes have been investigated (reviewed by Datta, 1966), little is known of the pharmacological action of drugs on cerebral ribonucleoprotein particles, which show marked alterations during experimental fatigue, exhaustion, stimulation etc. During these functional activities significant alterations occur in the distribution and staining properties of the RNA-rich basophilic Nissl granules (Einarson, 1932; Hyden, 1943, 1947; Krogh, 1950; Hyden & Larsson, 1956; Hyden & Pigon, 1960). On glucose-free perfusion for 50–60min about 50% of the microsomes disappear from the brain cortex, accompanied by a corresponding decrease of the nucleic acid content (Abood & Gieger, 1955). During electrical stimulation or under the action of metrazol, the amounts of cytoplasmic RNA-containing granules diminish (Gieger, 1956). Disaggregation of brain polyribosomes occurs in electroconvulsive treatment (Vesco & Giuditta, 1968). Previous studies in our laboratory have demonstrated that different convulsant drugs bring about significant changes in the ribonucleoprotein constituents of nerve tissue studied histologically and biochemically (Ghosh *et al.* 1962). It has been further observed that when brain-cortex slices are incubated in the presence of different convulsant and antidepressant drugs the ribosomal particles become more susceptible to degradation, releasing protein, RNA and acid-soluble nucleotides (Datta & Ghosh, 1963*a*, 1965; Ghosh *et al.* 1965). The results of the present study also indicate that mescaline induces some instability in the ribosomal particles and makes them susceptible to degradation into their components (Table 4 and Fig. 1). In its action on brain ribosomes mescaline may exert the same disorganizing effect as other convulsant and central-nervous-system-stimulant drugs.

The instability of ribosomes induced as a result of mescaline-treatment may account for the loss of specific activity of ribosomal enzymes (Table 2). Since these enzymes, like a few other enzymes of ribosomes from various sources, appear to be integral parts of brain-cortex ribosomes and cannot be removed by a number of drastic manipulations tried, a decrease in their specific activities due to drug treatment does reflect some alteration in the ribosomes themselves. Since the doses of mescaline used in the present study are within the pharmacological limits (Ghosh *et al.* 1962; Smythies *et al.* 1967), the effects observed may be definitive of mescaline action in respiring brain tissue. However, since mescaline is without

any inhibitory effect on the enzymic activities of freshly isolated normal brain-cortex ribosomes (Table 3), the primary effect of mescaline may not be on ribosomes. The changes observed in the ribosomes of mescaline-treated slices may reflect generalized cellular disorganization. Further study is desired to check whether other cellular organelles show the effects observed for ribosomes in the present study.

Further studies are also desirable to check whether the observed mescaline-induced instability of ribosomal particles is due to disturbance of ionic strengths or removal of binding metal ions and polyamines that normally stabilize the structural organization of ribonucleoprotein particles (Chao & Schachmann, 1956). Since the secondary structure plays an important role in maintaining the stability or integrity of both RNA and protein, an approach has been made in a subsequent investigation to study the molecular mechanism of disorganization of ribosomal particles isolated from brain-cortex slices previously treated with mescaline.

The stability of macromolecules is an important aspect of biological organization. This is especially true of ribosomes and other subcellular particles that constitute the principal components of intracellular biosynthetic systems. The instability induced in the ribosomes as a result of mescaline treatment of brain-cortex slices may lead to some impairment of this biosynthetic function of ribosomes. The relevance of the induced instability of ribosomes as observed in the present study to the pharmacological activities of mescaline-administration is yet to be established. It is not unlikely that the pharmacological activities of mescaline and other psychotomimetic drugs may be mediated through the specifically induced instability of ribosomes and perhaps other subcellular particles of brain tissue by these drugs.

Mescaline causes a disruption of the learned behaviours affecting both active and passive avoidance (Morpurgo, 1965). In view of the molecular theory of learning involving RNA molecules the mechanism of disruption of the learned behaviour by mescaline may be related to the induced instability of ribonucleoprotein particles of brain as a result of mescaline administration.

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