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MESCALINE-INDUCED CHANGES OF BRAIN CORTEX RIBOSOMES

EFFECT OF MESCALINE ON THE BINDING OF AMINOACYL-TRANSFER RIBONUCLEIC ACID TO RIBOSOMES OF BRAIN TISSUE*

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Abstract—The effect of mescaline sulfate on the binding of aminoacyl-tRNA by brain cortex ribosomes was studied. The poly U-directed binding of ^{14}C-phenylalanyl-tRNA *in vitro* was moderately inhibited by mescaline. The pretreatment of normal ribosomes with mescaline caused a decrease of their phenylalanyl-tRNA binding capacity. The pretreatment of brain cortex slices with mescaline also decreased the phenylalanyl-tRNA binding capacity of the ribosomes isolated from the slices so treated. This was true in assays with phenylalanyl-tRNAs from both *Escherichia coli* and yeast. This inhibitory effect was dependent on the concentration and length of treatment of brain cortex slices with mesaline.

THE TREATMENT of brain cortex slices, under respiring conditions, with mescaline (2,4,6-triethoxyphenylethylamine) sulfate decreases amino acid-incorporating abilities of ribosomes, when measured *in vitro*.[1] Such treatment also decreases the ribosomal synthesis of polyphenylalanine when assayed with synthetic messenger poly uridine (U) and *Escherichia coli* transfer RNA (tRNA).[2] However, mescaline treatment of brain cortex slices does not affect amino acid-binding properties of tRNAs or the activity of aminoacyl-tRNA synthetase.[3] In an attempt to elucidate the molecular mechanism of the mescaline-induced decrease of protein-synthesizing abilities of ribosomes, the present study compared the aminoacyl-tRNA binding capacities of ribosomes from untreated (glucose-treated) and from mescaline-treated (glucose + mescaline-treated) brain cortex slices. The capacities of ribosomes to bind aminoacyl-tRNA were measured in a clean, cell-free system in the presence of poly U, using ribosomes from brain cortex slices only. Purified *E. coli* ^{14}C-phenylalanyl-tRNA and synthetic poly U were added from outside. This measurement eliminated the participation of the mescaline-exposed cerebral tRNA and of messenger RNA (mRNA) in the ribosomal binding process. Results obtained by this measurement indicate that

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some decrease in the aminoacyl-tRNA binding capacities of ribosomes occurs as a result of mescaline treatment of brain cortex slices.

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 0·5 mm slices in the cold. Approximately 10 g wet weight of cortex slices was suspended in 100 ml Krebs–Ringer buffer (0·15 M NaCl, 3 mM KCl, 2 mM $CaCl_2$ and 0·2 M $NaHCO_3$) containing 0·5 M glucose, pH 7·0, previously saturated with O_2–CO_2 (95:5). Unless stated otherwise, the concentration of mescaline was 10 μg/g wet weight of brain cortex slices (or 1 μg/ml of the treatment medium). The respiring conditions of the slices were periodically checked by separate manometric experiments carried out under similar conditions. Mescaline had no effect on the respiration rate of the slices. After treatment at 37° for 2 hr the slices were collected by centrifugation and washed four times with 100-ml portions of Kreb–Ringer buffer. Unless stated otherwise, mescaline-treated cortex slices indicate glucose–mescaline treated slices, whereas the untreated (control) slices were treated with glucose alone.

Preparation of ribosomes. Ribosomes were prepared from slices with deoxycholate and purified by sucrose gradient centrifugation and characterized as described by Datta and Ghosh.[4,5]

Other preparations. The pH 5·0 enzymes were prepared as described by Datta and Ghosh.[1] Partially purified aminoacyl-tRNA synthetase was prepared as described by Datta and Antopol.[6] The acylation of *E. coli* tRNA with ^{14}C-phenylalanine was carried out according to the procedure of Zubay.[7] The product was further purified by DEAE Sephadex A-50 (Pharmacia) column chromatography according to the procedure of Cherayil and Bock[8] followed by absorption on DEAE cellulose and elution with NaCl (in urea) as described by Soll *et al.*[9] Commercially available extensively fractionated (according to Wimmer *et al.*[10]) and 22-fold purified brewer's yeast phenylalanine-tRNA (from Boehringer Manheim Corp., New York) was used to prepare ^{14}C-phenylalanyl-tRNA in a comparatively pure state.

Assay of aminoacyl-tRNA binding to ribosomes. The general procedure of Nirenberg and Leder[11] was used. Except where stated otherwise, the incubation system contained, in 0·05 ml: 5 μmoles Tris-acetate buffer, pH 7·2; 1 μmole magnesium acetate; 2·5 μmoles potassium chloride; 2·0 A_{260} units (optical density measurement in 1 ml with a 1-cm light path at 260 nm in 14 mM acetate veronal buffer, pH 7·0) of ribosomes, 20 nmoles uridylic acid residues in poly U and ^{14}C-aminoacyl-tRNA as shown in the tables. After incubation at 25° for 20 min, the reaction tubes were immediately cooled in ice and the contents mixed quickly with 3 ml of 0·1 M Tris-acetate buffer, pH 7·2, containing 0·2 M magnesium acetate and 0·05 M potassium chloride. The diluted reaction mixture was immediately poured on a cellulose nitrate filter (HA Millipore filter, 25 mm dia, 0·45 μ pore size) under suction. The filter was washed to remove unbound ^{14}C-aminoacyl-tRNA with three 3-ml portions of buffer at 0°. The dried filter was placed in vials containing scintillation solution and the radioactivity was determined in a liquid scintillation counter. The ^{14}C-amino acid bound to ribosomes was characterized by the procedure outlined by Nirenberg and Leder.[11] All assays were carried out in duplicate and results were accepted only when duplicates did not differ by more than 5 per cent.

Chemical determinations. Protein was determined by the method of Lowry *et al.*[12] with crystalline bovine serum albumin as a standard. RNA was determined by the orcinol method and also by an ultraviolet spectrophotometric procedure.

Materials. Crystalline mescaline sulfate was obtained from Hoffman–La Roche & Co. Ltd., Basel, Switzerland. It was neutralized before use. L-U-^{14}C-phenylalanine (345 mCi/m-mole) was purchased from Tracer Lab., Waltham, Mass, U.S.A. tRNA (sodium salt, stripped and free from ribonuclease) isolated from *E. coli* cells, strain B, was purchased from General Biochemicals, Chagrin Falls, Ohio, U.S.A.

RESULTS

Assay of ribosomal binding of ^{14}C-phenylalanyl-tRNA. The incubation of ribosomes from normal brain cortex slices with ^{14}C-phenylalanyl-tRNA and poly U resulted in the binding of ^{14}C-phenylalanyl-tRNA to ribosomes (Table 1, upper portion). Very little radioactivity was bound with ribosomes in the absence of poly U. Mg^{2+} was required for the binding, which proceeded linearly with time up to 25 min at 25°. Though the binding was maximal at 25°, it took place at a slower rate at lower temperatures.

According to Zomzely *et al.*,[13] the preincubation of ribosomes with postmicrosomal soluble supernatant or pH 5·0 enzymes leads to the destruction of endogenous cerebral mRNA by ribonuclease present in this fraction. As a check, purified ribosomes used in the present study were pre-incubated in the presence of post-microsomal soluble supernatant (105,000 *g*) and of pH 5·0 enzymes, collected by centrifugation and washed with buffer (Datta *et al.*[2]). A comparison of the pre-incubated ribo-

TABLE 1. ASSAY OF ^{14}C-PHENYLALANYL-tRNA BINDING TO RIBOSOMES OF NORMAL BRAIN CORTEX SLICES AND EFFECT OF MESCALINE *in vitro**

Modification	Mescaline added (μg/0·05 ml assay medium)	^{14}C-phenylalanyl-tRNA bound to 2·0 A_{260} units of ribosomes† (pmoles)	Inhibition (%)
Assay			
Complete system		2·02 ± 0·09	
Complete system minus ribosomes		0·03 ± 0·01	
Complete system minus poly U		0·04 ± 0·01	
Complete system minus ribosomes and poly U		0·02 ± 0·01	
Complete system minus magnesium acetate		0·30 ± 0·01	
Complete system minus potassium chloride		1·88 ± 0·07	
Effect of mescaline			
Complete system	0	2·00 ± 0·09	0
Complete system	0·1	1·94 ± 0·10	3
Complete system	0·3	1·90 ± 0·10	5
Complete system	0·5	1·80 ± 0·08	10
Complete system	1·0	1·68 ± 0·09	16
Complete system	1·5	1·61 ± 0·05	20
Complete system	2·0	1·50 ± 0·08	25
Complete system	2·5	1·39 ± 0·09	30

* Complete incubation system contained, in 0·05 ml: 5 μmoles Tris-acetate buffer, pH 7·2; 2·5 μmoles potassium chloride; 1 μmole magnesium acetate; 2·0 A_{260} units of ribosomes; 20 nmoles uridylic acid residues in poly U and 10 pmoles *E. coli* ^{14}C-phenylalanyl-tRNA (1520 cpm, 1·0 A_{260} unit). Other details are described in Methods and Materials. Values are given for three experiments using different ribosomal preparations.

† Values are means ± S.E.M.

somes with those not pre-incubated as stated above indicated no difference in their binding of ^{14}C-phenylalanyl-tRNA; both of them bound ^{14}C-phenylalanyl-tRNA equally in the presence of added poly U but failed to do so in the absence of poly U. This suggested that the purified ribosomes used in the present study were relatively free from endogenous mRNAs.

Effect of mescaline on the binding of ^{14}C-phenylalanyl-tRNA with normal ribosomes in vitro. The poly U-directed ribosomal binding of ^{14}C-phenylalanyl-tRNA was moderately inhibited by incubation with mescaline (Table 1, lower portion). The preincubation of ribosomes with mescaline for 30 min at 20° decreased further the binding activity of these ribosomes (when mescaline was not removed during the binding assay) (Table 2). The pretreatment of ribosomes with mescaline, which was later removed by centrifugation and two washings prior to the binding assay, had

TABLE 2. EFFECT OF PREINCUBATION OF RIBOSOMES WITH MESCALINE ON ITS BINDING OF *E. coli* ^{14}C-PHENYLALANYL-tRNA*

Preincubation	Mescaline removed before assay of binding	^{14}C-phenylalanyl-tRNA bound to 2·0 A_{260} units of ribosomes† (pmoles)	Inhibition (%)
None		1·98 ± 0·07	
Mescaline (1·0 μg)	No	1·58 ± 0·08	20
Mescaline (2·0 μg)	No	1·40 ± 0·04	30
Mescaline (1·0 μg)	Yes	1·64 ± 0·07	17
Mescaline (2·0 μg)	Yes	1·45 ± 0·06	27

* Freshly prepared ribosomes from normal brain cortex were preincubated with mescaline for 30 min at 20° in 0·05 ml Tris-acetate buffer (5 μmole), pH 7·2. The amounts of mescaline shown in the table are per 0·05 ml of preincubation medium. As shown in the table, mescaline was removed by centrifugation in some experiments. Thereupon, the rest of the incubation system as listed in Table 1 was added and the incubation was continued as described in Table 1 and in Methods and Materials. Values are given for three experiments using different ribosomal preparations.

† Values are means ± S.E.M.

TABLE 3. RELATIVE PHENYLALANYL-tRNA BINDING CAPACITIES OF RIBOSOMES FROM UNTREATED AND MESCALINE-TREATED BRAIN CORTEX SLICES*

Pretreatment	^{14}C-phenylalanyl-tRNA bound to 2·0 A_{260} units of ribosomes† (pmoles)	Inhibition (%)
E. coli ^{14}C-phenylalanyl-tRNA		
Glucose (0·5 M)	2·10 ± 0·08	
Glucose + mescaline‡	1·41 ± 0·07	33
Glucose + mescaline§	0·88 ± 0·07	58
Yeast ^{14}C-phenylalanyl-tRNA		
Glucose (0·5 M)	2·41 ± 0·11	
Glucose + mescaline‡	1·68 ± 0·08	30
Glucose + mescaline§	1·25 ± 0·07	48

* In binding experiments with *E. coli* aminoacyl-tRNA, 10 pmoles ^{14}C-phenylalanyl-tRNA (1520 cpm, 1·0 A_{260} unit) was included in the incubation system. In binding experiments with yeast aminoacyl-tRNA, 10 pmoles ^{14}C-phenylalanyl-tRNA (3105 cpm, 1·0 A_{260} unit) was included in the incubation system. Other details were the same as described in Table 1 and in Methods and Materials. Values are given for experiments using three different ribosomal preparations.

† Values are means ± S.E.M.

‡ Concentration was 5 μg/g wet wt of brain cortex slices or 0·5 μg/ml of treatment medium.

§ Concentration was 10 μg/g wet wt of brain cortex slices or 1 μg/ml of treatment medium.

also decreased the phenylalanyl-tRNA binding compared to that of ribosomes similarly pretreated without mescaline (Table 2). Hence it is concluded that mescaline has a direct inhibitory effect on the ribosomal binding process, which apparently remains even when mescaline is removed. Additional experiments involving pretreatment of poly U with mescaline indicated that mescaline had no effect on the activity of poly U in the binding process and did not affect the polyphenylalanine synthesis when measured with ribosomes from brain and liver tissues.

Binding capacities of ribosomes isolated from untreated and mescaline-treated brain cortex slices. The effect of mescaline treatment of brain cortex slices on the ^{14}C-phenylalanyl-tRNA binding capacities of ribosomes isolated from the treated and the untreated slices was determined. Results presented in Table 3 indicate that ribosomes isolated from the mescaline-treated slices have significantly less ^{14}C-phenylalanyl-tRNA binding capacity than those from the untreated slices. Results were similar with *E. coli* phenylalanyl-tRNA and yeast phenylalanyl-tRNA. This inhibitory effect on the ribosomal binding capacity was dependent on the concentration (Table 3) and length of the treatment of brain cortex slices with mescaline (Fig. 1).

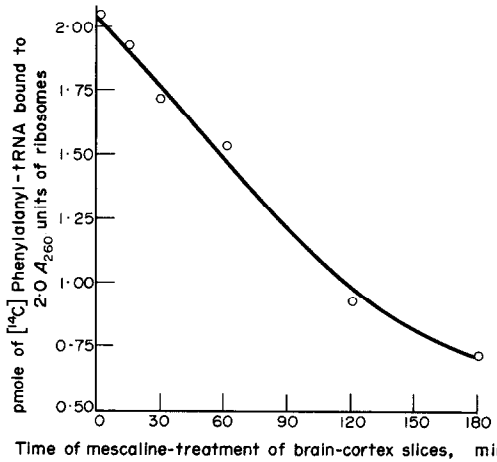

FIG. 1. Effect of time of mescaline treatment of brain cortex slices on the *E. coli* [^{14}C]phenylalanyl-tRNA binding capacity of ribosomes isolated from drug-treated slices. Treatment of brain cortex slices with mescaline (10 μg/g wet wt of brain cortex slices or 1 μg/ml of treatment medium) was described in Methods and Materials. Other details were the same as described in Table 1 and in Methods and Materials. Mean values of two experiments using different ribosomal preparations are plotted.

DISCUSSION

Experiments reported here were designed to resolve whether the mescaline-induced decrease in the protein-synthesizing ability of ribosomes was due to functional changes within the ribosomes themselves. The results, at least, indicated that mescaline treatment of brain cortex slices decreased the binding capacity of ribosomes for aminoacyl-tRNA. The decreased binding capacity of ribosomes from mescaline-treated slices was observed with ^{14}C-phenylalanyl-tRNA from two sources: *E. coli* and brewer's yeast (Table 3). Mescaline appeared to have an inhibitory effect on the ribosomal binding of aminoacyl-tRNA, since the decreased binding capacity

depended upon the concentrations of mescaline used (Table 3), and paralleled the duration of mescaline treatment (Fig. 1). This observation was strengthened by the finding that mescaline inhibited moderately the ribosomal binding of aminoacyl-tRNA *in vitro* and that this inhibitory effect remained even after mescaline was removed from the binding assay media (Tables 1 and 2). It is known that mescaline does not affect: (a) the physico-chemical properties of tRNAs, (b) the activities of aminoacyl-tRNA synthetases, (c) the activation of amino acids, (d) the acylation of tRNA or (e) the template activity of mRNA.[2,3] Therefore, it is suggested that the decreased aminoacyl-tRNA binding capacity of ribosomes may be one of the factors contributing to the mescaline-induced decrease of ribosomal protein synthesis observed previously by Datta and Ghosh.[1]

The molecular mechanism of the mescaline-induced decrease of ribosomal capacity to bind aminoacyl-tRNA needs further exploration. Axelrod[14] found that mescaline is demethylated by liver preparation. Such demethylation of mescaline also occurs in brain cortex slices, and ribosomes are appreciably methylated by a transmethylating enzyme present in the soluble supernatant (unpublished results). It may be likely that the methylation of ribosomes leads to the decrease of their aminoacyl-tRNA binding capacities.

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