MESCALINE-INDUCED CHANGES OF BRAIN CORTEX RIBOSOMES. EFFECT OF MESCALINE ON AMINO ACID INCORPORATING ABILITY OF RIBOSOMES

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INTRODUCTION

Brain ribosomes have fairly strong ability to incorporate labeled amino acids and behave in a manner greatly similar to liver ribosomes[1,15] (reviewed by Datta[7]). Several psychoactive drugs are known to influence protein synthesis of central nervous system (reviewed by Richter[25]). Krawczynski[14] observed that lysergic acid diethylamide (LSD) and 2-bromo-LSD inhibited cerebral protein synthesis *in vivo*. In view of these findings it was of interest to examine whether mescaline (3,4,5-trimethoxyphenethylamine) affects the protein synthesizing ability of brain cortex ribosomes. In previous studies, Datta and Ghosh[9,10] have demonstrated that mescaline acting on respiring brain cortex slices alters ribosomes by making them structurally unstable, and decreases the hydrogen bonded structures of ribosomal RNA. Since ribosomes are seats on which proteins are synthesized, any alteration, structural or otherwise, of ribosomes as a result of mescaline treatment may lead to altered protein synthesis. Hence attempts have been made to study (1) the *in vitro* effect of mescaline on amino acid incorporating ability of freshly prepared brain cortex ribosomes and (2) the effect of mescaline treatment of brain cortex slices on amino acid incorporating ability of the ribosomes isolated from slices so treated. The results presented in this communication indicate that mescaline causes a little inhibition of ribosomal incorporation of labeled amino acids and that ribosomes isolated from the mescaline-treated brain cortex slices have less ability to incorporate amino acids than those from the untreated control slices.

In a recent study, Datta and Ghosh[11] have found that polyamines counteract the destabilizing effect of mescaline on brain cortex ribosomes. Hence, in the present study polyamines have been included in some experiments to examine whether they can counteract the mescaline-induced decrease in amino acid incorporating abilities of ribosomes isolated from the drug-treated brain cortex slices. The results presented here indicate that polyamines are able to do so to some extent.

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 were suspended in 100 ml of Krebs–Ringer buffer (0.15 *M* NaCl, 3 m*M* KCl, 2 m*M* $CaCl_2$ 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 0.01 mg/g wet weight of brain cortex slices. The respiring conditions of the slices were periodically checked by separate manometric experiments carried out under similar conditions. After incubation at 37°C for 2 h the slices were collected by centrifugation and washed 4 times with 100 ml portions of Krebs–Ringer buffer. Unless otherwise stated, 'mescaline-treated' cortex slices indicate glucose–mescaline-treated slices, whereas 'untreated' (control) slices mean slices treated with glucose alone.

Treatment of brain cortex slices with spermidine

Same as in the previous section except that 50 m*M* spermidine was present during the treatment. Unless otherwise stated, 'spermidine-treated' cortex slices indicate glucose–spermidine-treated slices.

Treatment of brain cortex slices simultaneously with mescaline and spermidine

Same as in the previous section except that mescaline, 0.01 mg/g wet weight of brain cortex slices, and spermidine, 50 m*M*, were present during the treatment. Unless otherwise stated, 'mescaline–spermidine-treated' cortex slices indicate glucose–mescaline–spermidine-treated slices.

Preparation of ribosomes

Ribosomes were prepared from untreated and treated brain cortex slices by using deoxycholate as described by Datta and Ghosh[8]. Ribosomes were further purified by sucrose density gradient centrifugation and characterized as described by Datta and Ghosh[9]. In order to decrease the amounts of ribonuclease ribosomes were prepared in the presence of bentonite as described by Murthy and Rappoport[21].

Preparation of brain cortex soluble supernatant

The post-mitochondrial supernatant (15,000 × *g* of the brain cortex homogenate) was spun at 105,000 × *g* for 2 h. The supernatant was carefully pipetted out. This was then respun at 105,000 × *g* for 1 h and collected similarly. This soluble supernatant was used as such or passed through a column of Sephadex G-25 and eluted.

On some occasions, this was dialyzed overnight in the cold to remove amino acids, nucleotides and other small molecular weight substances.

Preparation of pH 5.0 enzymes

The brain soluble supernatant was adjusted to pH 5.0 with dropwise addition of 1 *N* acetic acid. A flocculent precipitate was collected by spinning at 105,000 × *g* for 2 h. The supernatant was also collected for use as *the pH 5.0-soluble fraction.* The precipitate which contained the pH 5.0 enzymes was solubilized in sucrose–phosphate (0.25 *M* sucrose, 0.1 *M* potassium phosphate), pH 5.5, before use in the incorporation studies.

Incorporation of [^{14}C]amino acids

Unless stated otherwise, the incubation medium contained 7.5 μmoles magnesium chloride, 2.5 μmoles ATP, 0.5 μmole GTP, 50 μmoles potassium chloride, 25 μmoles creatine phosphate, 0.08 mg creatine phosphokinase, 0.2 μmoles of uniformly labeled [^{14}C]leucine (5 μCi/μmole) (or other [^{14}C]amino acid), 75 μmoles Tris–HCl, pH 7.6, 0.5 mg ribosomal protein and 2.5 mg Sephadex-treated brain soluble supernatant (105,000 × *g*) or equivalent amounts of more purified preparation, in a total volume of 1 ml. Assay was carried out at 37°C for 30 min. The reaction was terminated by removing 0.1 ml aliquots to 3MM Whatman filter paper discs which were then dried under a heat lamp for 30 sec and dropped into a 5% TCA solution containing 0.25% sodium tungstate to increase the precipitation of small peptides[13]. The discs were washed 5 times with this TCA solution, dried and then counted in a scintillation counter. Proper controls were run and treated in the same way. As a check of the reliability of filter paper disc assay procedure, the incubation was stopped by adding 1 ml of 10% TCA solution (containing 0.25% sodium tungstate) into each tube and the mixtures were incubated for an additional period of 20 min at 95°C. The resulting hot TCA-insoluble materials were collected on Millipore filters (HA, pore size 0.45 μm) and radioactivity was counted in a scintillation counter. In both the procedures the results were fairly close. Moreover, in both the procedures all assays were carried out in duplicate and results were accepted only when the duplicates did not differ by more than 5%.

Chemical determinations

Protein was determined by the method of Lowry *et al.*[16], with crystalline bovine serum albumin as a standard. RNA was determined by the orcinol method and also by an UV-spectrophotometric procedure.

Materials

Spermidine trihydrochloride, spermine tetrahydrochloride, putrescine dihydrochloride and cadaverine dihydrochloride were purchased from Nutritional

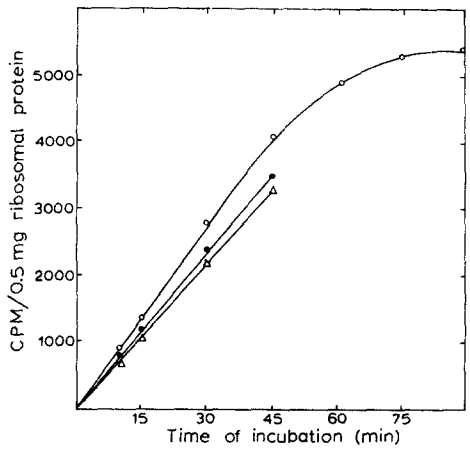

Fig. 1. The time-curve of [^{14}C]leucine incorporation into brain cortex ribosomes in the absence or in the presence of mescaline. The incorporation system was as described in Table I and in Methods and Materials . ○, No mescaline; ●, mescaline (5 μg); △, mescaline (10 μg).

Biochemicals Corp., Cleveland, Ohio, U.S.A. They were neutralized before use.

Crystalline mescaline sulfate was obtained from Hoffmann–La Roche and Co. Ltd., Basel, Switzerland. This was neutralized before use.

L-[U-^{14}C]Leucine (248 mCi/mmole), L-[U-^{14}C]arginine (210 mCi/mmole) and L-[U-^{14}C]phenylalanine (345 mCi/mmole) were purchased from Tracer Lab., Waltham, Mass., U.S.A.

RESULTS

Characteristics of [^{14}C]leucine incorporation into brain cortex ribosomes

Preliminary studies indicated that the pH for maximal incorporation of [^{14}C]-leucine into brain ribosomes was between 7.4 and 7.8. The rate of incorporation was proportional up to 45 min and became almost stationary in 75 min (Fig. 1). The 105,000 $\times$ g brain cortex soluble supernatant was needed for the incorporation. However, this supernatant contained some inhibitory substances which decreased the incorporation rate. The inhibitory substances were removed by gel filtration of the supernatant through Sephadex G-25 and partially by dialysis. The [^{14}C]leucine incorporation into brain cortex ribosomes was 40–45 pmoles of leucine incorporated/mg of ribosomal protein/min when assayed by the procedure as described in Methods and Materials. Results of a typical assay are tabulated in Table I. Both ATP-generating system and GTP were needed for maximal incorporation of leucine.

Effect of mescaline on the [^{14}C]leucine incorporation into freshly isolated brain cortex ribosomes

Ribosomes were isolated and purified from freshly obtained normal brain cortex.

TABLE I

[^{14}C]LEUCINE INCORPORATION INTO BRAIN CORTEX RIBOSOMES

Complete incorporation system contained 7.5 μmoles $MgCl_2$, 2.5 μmoles ATP, 0.5 μmole GTP, 50 μmoles KCl, 25 μmoles creatine phosphate, 80 μg creatine phosphokinase, 0.2 μmole uniformly labeled [^{14}C]leucine, 75 μmoles Tris–HCl, pH 7.6, ribosomes containing 0.5 mg ribosomal protein and Sephadex-treated brain cortex soluble supernatant (105,000 $\times$ *g*) containing 2.5 mg protein in a total volume of 1 ml. Other details were as described in Methods and Materials.

Incorporation system	*Counts/min/0.5 mg ribosomal protein/30 min*	*pmoles of [^{14}C]leucine incorporated/mg ribosomal protein/min*
Complete system	2910	45
Complete system, minus GTP	121	2
Complete system, minus soluble supernatant	75	1
Complete system, minus ATP-generating system	73	1

TABLE II

In vitro EFFECTS OF MESCALINE AND SPERMIDINE ON THE INCORPORATION OF [^{14}C]LEUCINE INTO RIBOSOMES FRESHLY ISOLATED FROM NORMAL BRAIN CORTEX

The incorporation system was same as described in Table I and in Methods and Materials. Values of experiments using 3 ribosomal preparations are given.

Mescaline (μg)	*Spermidine (μmole)*	*pmoles of [^{14}C]leucine incorporated/mg ribosomal protein/min (mean $\pm$ S.E.M.)*	*Mean activity (%)*
0	0	45.1 $\pm$ 2.0	100
4	0	42.6 $\pm$ 2.5	94
6	0	40.3 $\pm$ 1.6	89
10	0	38.5 $\pm$ 2.1	85
25	0	37.0 $\pm$ 1.5	82
0	5	47.0 $\pm$ 2.7	104
0	10	47.8 $\pm$ 2.3	106
10	5	43.2 $\pm$ 1.4	96
10	10	44.0 $\pm$ 1.9	98

These ribosomes were found to be relatively free from endogenous ribonuclease activity when incubated in the presence of yeast RNA. These ribosomes were used to study the *in vitro* effect of mescaline on the ribosomal incorporation of [^{14}C]leucine. Results presented in Table II indicate that mescaline, at a concentration up to 10 μg/mg ribosomal protein, caused a slight inhibition of ribosomal incorporation of [^{14}C]-leucine, and this inhibitory effect was dependent on the concentrations of mescaline present in the incorporation medium. Addition of spermidine during ribosomal incorporation of labeled amino acids did not significantly increase the rate of incorpora-

TABLE III

EFFECTS OF PREINCUBATION OF RIBOSOMES WITH MESCALINE AND SPERMIDINE ON THE [^{14}C]LEUCINE INCORPORATION INTO BRAIN CORTEX RIBOSOMES

Ribosomes freshly isolated from normal brain cortex were suspended in buffer and incubated in the presence of mescaline, spermidine and 105,000 × *g* soluble supernatant for 30 min. Thereupon, the rest of the incorporation system as listed in Table I and in Methods and Materials was added to the preincubated ribosomes and the incubation was continued for 30 min. Values of experiments using 3 ribosomal preparations are given.

Preincubation of freshly isolated ribosomes with	*pmoles of [^{14}C]-leucine incorporated/mg ribosomal protein/min (mean ± S.E.M.)*	*Mean activity (%)*
None	44.2 ± 2.2	100
Mescaline (5 μg)	41.0 ± 1.8	93
Mescaline (10 μg)	36.0 ± 2.9	81
Spermidine (5 μmoles)	47.5 ± 2.0	107
Mescaline (10 μg) plus spermidine (5 μmoles)	45.1 ± 1.8	102
Mescaline (5 μg) plus soluble supernatant (2.5 mg)	40.1 ± 3.0	91
Mescaline (10 μg) plus soluble supernatant (2.5 mg)	34.1 ± 1.7	77

tion (Table II). Similar was the effect of addition of spermine. When both mescaline and spermidine were added to the incorporation media, the mescaline-induced inhibition of ribosomal incorporation of amino acids was mostly counteracted (Table II). As shown in Fig. 1 the inhibitory effect of mescaline was time-dependent. Further, the inhibitory effect was slightly higher (1) when the ribosomes were preincubated for 30 min in the presence of mescaline, and (2) when the ribosomes were preincubated in the presence of mescaline and 105,000 × *g* soluble supernatant for 30 min before addition of the incorporation media (Table III). Quantitatively, similar inhibition (10–15%) was observed when the ribosomal incorporation of [^{14}C]phenylalanine and [^{14}C]arginine was measured in the presence of mescaline under similar conditions. Addition of spermidine during pretreatment of ribosomes caused a slight increase in the rate of ribosomal incorporation of labeled leucine (Table III). Similar was the effect of addition of spermine. When both mescaline and spermidine were added during pretreatment of ribosomes, the mescaline-induced inhibition of ribosomal incorporation of amino acids was mostly counteracted (Table III).

Effect of mescaline treatment of brain cortex slices on the [^{14}C]leucine incorporating ability of ribosomes isolated from drug-treated slices

In view of the above slight *in vitro* inhibitory effect of mescaline on the incorporation of [^{14}C]leucine and two other amino acids into brain cortex ribosomes in a cell-free system, and of the previous finding that mescaline treatment affected ribosomes structurally by making ribosomes susceptible to breakdown and decreasing ribosomal

TABLE IV

[^{14}C]LEUCINE INCORPORATION INTO RIBOSOMES ISOLATED FROM UNTREATED (CONTROL), MESCALINE-TREATED, SPERMIDINE-TREATED AND MESCALINE–SPERMIDINE-TREATED BRAIN CORTEX SLICES

Treatment of brain cortex slices with mescaline, spermidine and their combination was described in Methods and Materials. The [^{14}C]leucine incorporation system was same as described in Table I. Values of experiments using 3 different ribosomal preparations are given.

Ribosomes isolated from brain cortex slices previously treated with	*pmoles of [^{14}C]leucine incorporated/mg ribosomal protein/min (mean ± S.E.M.)*	*Mean activity (%)*
None (0.5 *M* glucose)	40.2 ± 2.3	100
Mescaline (5 μg/g wet weight of brain cortex slices)	32.4 ± 3.1	81
Mescaline (10 μg/g wet weight of brain cortex slices)	25.6 ± 3.2	64
Spermidine (50 m*M*)	42.0 ± 1.7	104
Mescaline (10 μg/g wet weight of brain cortex slices) plus spermidine (50 m*M*)	36.5 ± 3.3	91

RNA hydrogen bonds[9,10], it was of interest to examine the effect of mescaline treatment of brain cortex slices on the amino acid incorporating ability of ribosomes isolated from the slices so treated. As described in Methods and Materials, freshly obtained brain cortex slices were treated with mescaline in suspension in Krebs–Ringer buffer, pH 7.0, containing 0.5 *M* glucose at 37°C for 2 h, the respiring conditions of the slices having been periodically checked by separate manometric experiments carried out under similar conditions. Ribosomes were prepared from the drug-treated slices, purified and used in amino acid incorporation studies. The ribosomes isolated from the mescaline-treated brain cortex slices had less [^{14}C]leucine incorporating ability than those from the untreated control slices (Table IV). In other words, the mescaline treatment of brain cortex slices resulted in the decreased amino acid incorporating ability of ribosomes isolated from the slices so treated. This inhibitory effect of mescaline treatment was dependent on the concentrations of mescaline present during mescaline treatment; a 19% inhibition of [^{14}C]leucine incorporation occurred when mescaline was 5 μg/g wet weight of brain cortex slices, whereas a 36% inhibition of [^{14}C]leucine incorporation occurred when the mescaline concentration was doubled. Ribosomes isolated from the spermidine-treated brain cortex slices had slightly greater [^{14}C]leucine incorporating ability than those from the untreated control slices. However, when spermidine was present during the mescaline treatment, the inhibition of [^{14}C]leucine incorporation by mescaline–spermidine-treated ribosomes was less than that by the mescaline-treated ribosomes, suggesting that spermidine protected, to some extent, the inhibitory effect of mescaline on the amino acid incorporating ability of such ribosomes (Table IV).

At mescaline concentration of 10 μg/g wet weight of brain cortex slices, the amino acid-incorporating ability of ribosomes of drug-treated slices towards incorporation of [^{14}C]phenylalanine and [^{14}C]arginine was also low compared to that of the

TABLE V

EFFECT OF TIME OF MESCALINE TREATMENT OF BRAIN CORTEX SLICES ON THE [^{14}C]LEUCINE INCORPORATING ABILITY OF RIBOSOMES ISOLATED FROM THE SLICES

Treatment of brain cortex slices with mescaline was described in Methods and Materials. The concentration of mescaline was 10 μg/g wet weight of brain cortex slices. The [^{14}C]leucine incorporation system was same as described in Table I. Average values of experiments using two different ribosomal preparations are given.

Time of mescaline treatment of brain cortex slices (min)	*pmoles of [^{14}C]leucine incorporated/mg ribosomal protein/min*	*Inhibition (%)*
0	41.3	—
15	38.0	8
30	33.4	19
60	28.9	30
120	24.9	40
180	20.0	52

ribosomes of the untreated control slices. As presented in Table V the inhibitory effect of mescaline treatment on the amino acid incorporating ability of ribosomes of drug-treated slices was time-dependent.

DISCUSSION

Results presented in previous sections indicate that on the basis of incorporation of amino acids by brain cortex ribosomes in cell-free systems, mescaline decreases slightly the *in vitro* amino acid incorporating ability of ribosomes freshly prepared from normal brain cortex slices (Table II). This inhibitory effect of mescaline on amino acid incorporating ability becomes greater when the brain cortex slices are treated with mescaline prior to the isolation of ribosomes from the slices so treated (Table IV). This inhibitory effect of mescaline treatment was dose- and time-dependent (Tables IV and V). Similar inhibitory effects of mescaline on the poly-U directed polyphenylalanine synthesis by brain cortex ribosomes have been recently observed (Datta and Ghosh, unpublished results). The inhibitory effect of mescaline on the protein synthesizing ability of brain cortex ribosomes is comparable to the effects of some narcotic drugs such as morphine and some of its derivatives. Clouet and Ratner[6] observed that following injections of morphine in rats, brain ribosomes had a decreased ability to incorporate labeled amino acids into protein *in vitro*, significantly different from the control level. Earlier, Clouet and Ratner[5] observed that, in morphine-treated rats, following injection of labeled leucine directly into the cerebrospinal fluid, there was a significant inhibition of leucine incorporation into protein of the microsomal and soluble fractions of rat brain with maximal inhibition at 2–3 h after a single injection of morphine. Further, in *E. coli* grown in the presence of levorphanol[26] and in HeLa cells grown in the presence of levallorphan[24], protein synthesis was inhibited in the presence of the aforesaid narcotic drugs. Though there is no known rele-

vance of the observed decreased amino acid incorporating ability of ribosomes as a result of mescaline treatment to the pharmacological activities of mescaline, attempts are now in progress to explore the mechanism of how mescaline decreases the amino acid incorporating ability of ribosomes of brain cortex slices.

The inhibitory effect of mescaline on the leucine incorporating ability of freshly isolated normal brain cortex ribosomes has been shown to be slightly higher (1) when the ribosomes are preincubated in the presence of mescaline, and (2) when the ribosomes are preincubated in the presence of mescaline plus the 105,000 $\times$ g brain cortex soluble supernatant (Table III). This observation together with the finding that mescaline when present in the incorporation medium causes a slight inhibition of ribosomal incorporation of leucine (Table II) suggests that mescaline may have some direct effect on the ribosomal particles, presumably either by its binding with the ribosomes, or by altering the ribosomes structurally or by chemically modifying the ribosomes. The possibility that mescaline binds with ribosomes has recently been observed (Datta and Ghosh, unpublished results). The possibility that mescaline may cause structural alterations of ribosomes has been demonstrated; during the action of mescaline on brain cortex slices the ribosomal particles become susceptible to breakdown, releasing protein, RNA and acid-soluble nucleotides[9]. Axelrod[2] found that mescaline is demethylated by rabbit liver preparations. Such demethylation of mescaline also takes place in brain cortex slices and ribosomes are appreciably methylated and the transmethylating enzyme is present in the soluble supernatant (Datta and Ghosh, unpublished results). In view of this finding, it is likely that when preincubated with mescaline in the presence of the 105,000 $\times$ g soluble supernatant ribosomes are slightly more methylated than in the absence of soluble supernatant, leading to a greater decrease in their amino acid incorporating ability (Table III).

Studies by various investigators[3,12,17,20,27,29] have indicated that spermidine, spermine and other polyamines are generally effective in preserving ribosomes from different sources. Datta and Ghosh[11] demonstrated recently that mescaline treatment of brain cortex slices removed a portion of endogenous spermidine from ribosomes and that polyamines were effective in counteracting the destabilizing effect of mescaline on ribosomes. In good agreement with these observations is the finding of the present study that spermidine protects, to some extent, the inhibitory effect of mescaline on the amino acid incorporating ability of ribosomes (Table IV). This observation is quite relevant to the observation that polyamines stimulate incorporation of amino acids into polypeptides *in vitro*[4,18,19,22,23,28].

SUMMARY

Mescaline, at a concentration up to 10 μg/mg ribosomal protein, caused a slight inhibition of incorporation of [^{14}C]leucine, arginine and phenylalanine into ribosomes freshly prepared from normal brain cortex slices. Spermidine or spermine did not significantly increase the rate of incorporation. However, spermidine present in the incorporation media counteracted mostly the mescaline-induced inhibition of ribosomal incorporation of amino acids. The treatment of brain cortex slices under respir-

ing conditions with mescaline (5–10 μg/g wet weight of brain cortex slices) resulted in marked decreases in the amino acid incorporating ability of ribosomes isolated from the slices so treated. This inhibitory effect of mescaline treatment was concentration- and time-dependent. However, when spermidine was present during the mescaline treatment of brain cortex slices the mescaline-induced inhibition of ribosomal amino acid incorporation was significantly counteracted.

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