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# FATE OF mRNA FOLLOWING DISAGGREGATION OF BRAIN POLYSOMES AFTER ADMINISTRATION OF (+)-LYSERGIC ACID DIETHYLAMIDE IN VIVO *

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## Summary

Intravenous injection of (+)-lysergic acid diethylamide into young rabbits induced a transient brain-specific disaggregation of polysomes to monosomes. Investigation of the fate of mRNA revealed that brain poly(A+)mRNA was conserved. In particular, mRNA coding for brain-specific S100 protein was not degraded, nor was it released into free ribonucleoprotein particles. Following the (+)-lysergic acid diethylamide-induced disaggregation of polysomes, mRNA shifted from polysomes and accumulated on monosomes. Formation of a blocked monosome complex, which contained intact mRNA and 40-S plus 60-S ribosomal subunits but lacked nascent peptide chains, suggested that (+)-lysergic acid diethylamide inhibited brain protein synthesis at a specific stage of late initiation or early elongation.

## Introduction

The complexity of transcription of nonrepeated DNA in mammalian brain tissue has previously been analysed by RNA/DNA hybridization [1—4]. Transcription of nonrepeated DNA in brain increases during early postnatal development [3,4] at a time when the rate of protein synthesis dramatically declines

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Abbreviations: eIF-2, eukaryotic initiation factor forming ternary complex with GTP and initiator Met-tRNA; EF-1 and EF-2, elongation factors 1 and 2; SDS, sodium dodecyl sulfate; LSD, (+)-lysergic acid diethylamide.

[5—8]. These observations of an increased transcriptional complexity and a decline in the translational rate during early postnatal development raise questions as to how protein synthesis in the brain is regulated and whether control mechanisms are similar to those operating in other cell types.

We have used the drug, (+)-lysergic acid diethylamide (LSD) to alter biochemical processes experimentally in the brain in an attempt to elucidate brain specific regulatory mechanisms. We have reported that LSD stimulated the acetylation of specific brain histones [9], increased the ability of isolated brain nuclei to synthesize RNA [10] and decreased in vivo incorporation of [$^{35}$S]-methionine into brain protein via a polysome disaggregation mechanism [11]. The disaggregation of polysomes, which is brain specific, was mediated by the interaction of LSD with neurotransmitter receptors [12] and the extent of the disaggregation increased with age and stress [13,14]. We have previously shown that the increase in brain monosomes after LSD administration is not the result of either RNAase degradation of polysomes or premature ribosome 'fall-off' [13].

In this report we analyse the fate of mRNA following LSD-induced brain polysome disaggregation. We find that there is no detectable decrease in the amount of brain mRNA following LSD administration and that intact mRNA is found associated with 40S and 60S ribosomal subunits as a blocked initiation complex. The majority of investigations on translational control mechanisms have been carried out on single cells such as reticulocytes [15—18] and Ehrlich ascites cells [19,20]. This study is of particular interest since we analyse the regulation of protein synthesis in a complex multicellular organ in which a psychotropic drug interacts with neurotransmitter receptors leading to a transient brain-specific inhibition of protein synthesis.

## Materials and Methods

*Drug administration.* Young male New Zealand White rabbits weighing 1.2—1.4 kg were kept on a 12 h light-dark cycle and fed ad lib. LSD (Sandoz Pharmaceuticals, Switzerland) obtained from the Department of National Health and Welfare, Ottawa, Canada, was dissolved in 0.9% NaCl and injected into a marginal ear vein at a dose of 100 μg/kg. Control animals received an equal volume of saline. After 30 min, animals were killed by cervical dislocation and the brains were removed.

*Isolation of polysomes and ribonucleoprotein particles.* Cerebral hemispheres were homogenized in 0.32 sucrose containing 50 mM Tris-HCl, pH 7.6, 25 mM KCl, 5 mM KCl, 5 mM $MgCl_2$ and 2 mM dithiothreitol, and RNA was isolated from purified polysomes isolated as previously described [14,22]. Subribosomal ribonucleoprotein (RNP) particles were isolated by a modification of the method of Bag and Sarker [23] as follows. Cerebral hemispheres were homogenized in 3 vols. of the same homogenization buffer used above and centrifuged for 10 min at 11 500 rev./min in the Sorvall SS-34 rotor. The postmitochondrial supernatant was centrifuged for 1 h at 37 500 rev./min in a Beckman 60 Ti rotor at 4°C. The supernatants were collected and diluted with 2 vols. of homogenization buffer minus sucrose and centrifuged for 3 h at 50 000 rev./min in a Beckman 60 Ti rotor. The postribosomal supernatants were

adjusted to 0.5 M KCl and centrifuged for 12 h at 34 000 rev./min in a Beckman 60 Ti rotor. The pelleted RNP particles were suspended in pellet buffer and RNA was extracted by the SDS-phenol method as previously described for polysomal RNA [22].

*Sucrose density gradient centrifugation of polysomes and cytoplasmic extracts.* Polysomes (30 $A_{260}$ units) were suspended in homogenization buffer minus sucrose and layered onto 15—40% (w/v) linear sucrose gradients in the same buffer. Gradients were centrifuged for 3 h at 26 000 rev./min in a Beckman SW-27 rotor at 4°C. The gradients were displaced upwards with dense sucrose, monitored continuously at 254 nm and fractions collected as indicated by the horizontal bars in the figures. Ribosomes were collected by centrifugation for 12 h at 30 000 rev./min in a 60 Ti rotor.

Postmitochondrial extracts were prepared by homogenization of cerebral hemispheres in 3 vols. of homogenization buffer and centrifugation for 10 min at 11 500 rev./min in the Sorvall SS-34 rotor. Postmitochondrial supernatants were layered onto 15—40% (w/v) linear sucrose gradients containing homogenization buffer minus sucrose and centrifuged for 4 h at 26 000 rev./min in a Beckman SW-27 rotor at 4°C. Gradients were fractionated as described above and the fractions precipitated with 2.5 vols. cold ethanol overnight. The RNA in each fraction was purified by phenol extraction [22] and assayed for poly(A+)mRNA by hybridization with [$^3$H]polyuridylate.

*Quantitation of polyadenylated mRNA.* Polyadenylated mRNA was quantitated by hybridization with [$^3$H]polyuridylate as described by Rosbash and Ford [24]. Purified RNA (10—50 $\mu$g) was dissolved in 0.5 ml twice standard saline-citrate solution (0.3 M NaCl, 0.03 M sodium citrate, pH 8.0), [$^3$H]-polyuridylate (0.05 $\mu$Ci in 20 $\mu$l; 18 Ci/mol of polynucleotide phosphorus, Schwartz Mann) was added and the mixture was incubated 30 min at 45°C, then chilled on ice. Ribonuclease A (50 $\mu$g in 2 ml twice standard saline-citrate solution) was added and the mixture incubated for 20 min at 4°C. The mix was adjusted to 5% trichloroacetic acid and carrier bovine serum albumin (500 $\mu$g) was added. After 1 h the precipitates were collected on pre-soaked Millipore filters, solubilized with 1 ml Soluene 100 (Packard) and counted in a PPO/POPOP/toluene scintillation mixture.

*Cell-free protein synthesis with wheat embryo extracts.* A wheat embryo extract (30 000 $\times g$ supernatant) was prepared as described previously [22] for the translation of poly(A+)mRNA and the LSD-induced blocked initiation complex. [$^{35}$S]methionine (New England Nuclear) was used at a final concentration of 200 $\mu$Ci/ml and the reaction mixtures (250 $\mu$l) were incubated at 24°C for 30 min. Aliquots of the reaction mixture (25 $\mu$l) were precipitated by addition of 5% trichloroacetic acid and the precipitates were trapped on presoaked Millipore filters. Filters were washed twice with 4 ml 5% trichloroacetic acid and once with 4 ml chloroform/ethanol (1 : 1, v/v), dried under a heat lamp and counted in a toluene scintillation mixture. Incorporation at zero time was subtracted from all points.

*Measurement of the mRNA coding brain-specific S100 protein.* S100 protein mRNA activity was quantitated by measuring the synthesis of S100 protein in a wheat embryo cell-free system programmed with brain mRNA isolated either from polysomes or RNP particles. S100 protein synthesis was determined by

immunoprecipitation of released peptides with monospecific anti-S100 protein anti-serum and electrophoresis in polyacrylamide gels as we have previously described [26,27]. The relative synthesis of S100 protein is (radioactivity in S100 protein)/(radioactivity in released peptides) × 100. The specific activity of S100 protein mRNA is represented by the radioactivity in S100 protein/$\mu$g mRNA used to program the reaction.

## Results

*Quantitation of brain mRNA following polysome disaggregation by LSD in vivo*

Our previous work [11,13] has demonstrated that the intravenous injection of LSD to young adult rabbits induced a transient, brain-specific disaggregation of polysomes to monosomes. Analysis of the high salt dissociability and tRNA content of the large monosome peak which was generated following drug administration indicated that the polysome disaggregation was not a result of RNAase activation [13]. Brain mRNA might however, be degraded by nuclease following the disaggregation event necessitating the synthesis of new mRNA for polysome reformation. We have now addressed ourselves to the question of determining the fate of brain mRNA following brain polysome disaggregation.

Ribonuclease activity was measured in brain postmitochondrial supernatants from control and LSD-treated animals (100 $\mu$g/kg for 30 min). No significant change was observed in the levels of either alkaline ribonuclease activity assayed at pH 7.4 in the presence of 12 mM $Mg^{2+}$ ion or acid ribonuclease II activity assayed at pH 5.8 in the absence of $Mg^{2+}$ [21]. This result is consistent with our previous conclusion of a non-RNAase mechanism for the LSD-induced polysome disaggregation [11,13].

Further support for this conclusion was gained by measuring the amount of poly(A+)mRNA in brain extracts from control and LSD-treated animals (100 $\mu$g/kg for 30 min). Since the majority of mRNA molecules are polyadenylated [28], brain mRNA was quantitated by hybridization to [$^3$H]polyuridylate as described in Materials and Methods. Equal amounts of poly(A+)mRNA were found associated with either postmitochondrial supernatant extracts isolated from control and drug-treated animals (i.e. 1045 and 1081 cpm/$\mu$g RNA respectively) or polysomes (i.e. 1323 and 1610 cpm/$A_{260}$ respectively). This result suggested that brain mRNA was not degraded when protein synthesis was inhibited by LSD. The slight increase in the amount of mRNA associated with polysomes from LSD-treated animals probably reflected a small loss of ribosomal subunits into a pool of inactive ribosomes [29].

Binding of polyadenylated mRNA to oligo(dT)-cellulose was used to provide a more sensitive determination of whether mRNA was nicked following LSD administration. Brain RNA was labelled in vivo by the intraventricular injection of 1 mCi [$^3$H]uridine. After 30 min, animals were injected intravenously (100 $\mu$g/kg) with LSD or saline (control) and killed 30 min later. The percentage of pulse-labelled RNA binding to oligo(dT)-cellulose was not affected by LSD treatment, suggesting that LSD did not produce cleavage of brain mRNA in either the cerebral hemispheres (control 22.7%, LSD 23.1%) or brain stem plus cerebellum (control 27.9% and LSD 28%).

*Intracellular distribution of brain mRNA following LSD-induced polysome disaggregation*

An LSD-induced shift of polysomes to monosomes with a concomitant release of mRNA might be expected to alter the intracellular distribution of rapidly labelled RNA. The distribution of pulse-labelled RNA was therefore examined in the nuclear, mitochondrial, polysomal and postribosomal supernatant fractions of the cerebral hemisphere before and after LSD administration (100 μg/kg for 30 min) and no overall change was detected (1 mCi [$^3$H]-uridine was injected into the lateral ventricles of the brain 1 h prior to LSD).

Since mRNA might be released from polysomes and appear in the postribosomal supernatant as RNP particles, the cytoplasmic distribution of mRNA coding for the brain-specific S100 protein was analyzed. Cytoplasmic RNP particles were isolated from control and LSD-treated animals, and mRNA purified from these fractions was translated in a wheat embryo cell-free system. Newly synthesized released proteins were analyzed for the presence of S100 protein by our previously described immunoprecipitation assay [22,27]. The results revealed no increase in mRNA coding for S100 protein in RNP particles following LSD treatment (Table I). When this same assay was used to analyse the level of mRNA for S100 protein which was associated with brain polysomes at various time points during the disaggregation event, there was no significant change in the level of mRNA coding for this protein compared to controls (Table II). It should be noted that in this experiment the polysome p[illegible]arations included monosomes. As shown in the following experiments, this point was critical to comprehending the fate of brain mRNA following LSD-induced polysome disaggregation.

*Shift of brain mRNA from polysomes to monosomes after LSD administration*

The following considerations, i.e. no RNAase degradation of brain mRNA, no release of mRNA coding for S100 protein into the cytosol as RNP particles and no change in the level of mRNA for S100 protein which was associated with ribosomes during the course of a major polysome disaggregation event, suggested that relocalization of mRNA between polysomes and monosomes might occur following LSD administration. This possibility was examined in

TABLE I

EFFECT OF LSD ON THE LEVEL OF S100 PROTEIN mRNA ACTIVITY IN FREE RNP PARTICLES

RNP particles were prepared from three control and three LSD-treated (100 μg/kg for 30 min) animals. RNA was isolated by phenol extraction, heat denatured in the presence of formaldehyde and translated in wheat embryo extracts. Synthesis of S100 protein was measured by immunoprecipitation and polyacrylamide gel electrophoresis. Relative synthesis of S100 protein is (radioactivity in S100 protein)/(radioactivity in released peptides) X 100. Specific activity of S100 protein mRNA is radioactivity in S100 pro[illegible]er μg mRNA used to program reaction. Data represent means of three experiments each for control and LSD-treated animals. Actual values were: control, 712 cpm and LSD, 795 cpm into S100 protein.

| Parameter measured | Treatment | | Ratio, drug/control |
|---|---|---|---|
| | Control | LSD | |
| Relative synthesis of S100 protein | 0.11 | 0.12 | 1.09 |
| Specific activity of S100 protein mRNA | 14 | 15 | 1.07 |

TABLE II

EFFECT OF LSD ON THE LEVEL OF S100 PROTEIN mRNA ACTIVITY IN POLYSOMES

Polysomal poly(A+)mRNA was purified from control and drug-treated (100 μg/kg) animals at various times after LSD administration and translated in wheat embryo extracts. Synthesis of S100 protein was determined by immunoprecipitation and polyacrylamide gel electrophoresis. The relative synthesis of S100 protein is (radioactivity in S100 protein)/(radioactivity in released peptides) X 100. The specific activity of S100 protein mRNA is radioactivity in S100 protein/μg mRNA used to program reaction. The values shown represent the mean values of the number of experiments in parentheses.

| Time after LSD administration (min) | Relative synthesis of S100 protein (%) | Specific activity of S100 protein mRNA |
|---|---|---|
| 0 | 0.25 | 85 (9) |
| 30 | 0.24 | 94 (2) |
| 45 | 0.17 | 84 (2) |
| 60 | 0.17 | 81 (3) |
| 360 | 0.24 | 94 (1) |

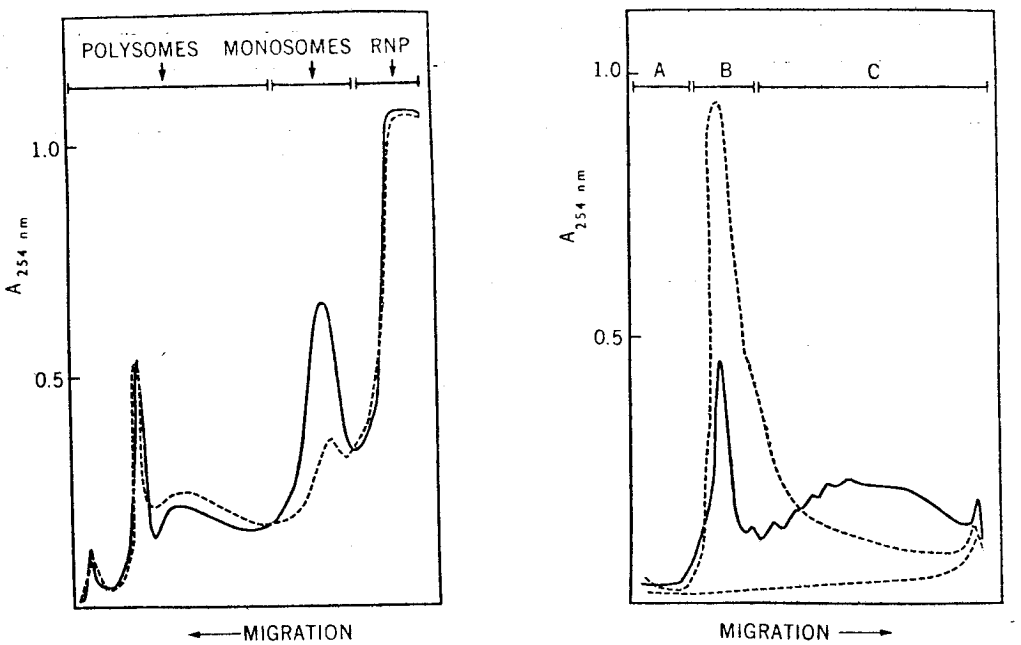


Fig. 1. Sucrose gradient fractionation of cytoplasmic extracts from control and LSD-treated animals. Postmitochondrial supernatants were prepared from brains of animals injected with saline (- - - - - -) or LSD (———) (100 μg/kg for 30 min). Aliquots were layered onto 15—40% (w/v) linear sucrose gradients and centrifuged for 4 h at 26 000 rev./min in the SW-27 rotor at 4°C. The gradients were displaced upward with dense sucrose and the absorbance monitored continuously with LKB-Uvicord II at 254 nm. The gradients were fractionated as indicated by the horizontal bars and each fraction precipitated overnight with 2 vols. cold ethanol for phenol extraction of RNA. The sharp peak near the bottom of the gradient represents membranous elements including synaptosomes.

Fig. 2. Fractionation of purified polysomes from control and LSD-treated animals. Brain polysomes were isolated from saline and drug treated (100 μg/kg for 30 min) animals and layered onto 15—45% (w/v) linear sucrose gradients. Centrifugation was for 3 h at 26 000 rev./min in the SW-27 rotor at 4°C. Gradients were displaced upwards with dense sucrose and monitored continuously at 254 nm. The gradients were fractionated as shown by the horizontal bars and the ribosomes pelleted overnight at 30 000 rev./min in the 60 Ti rotor. (———), 32 $A_{260}$ units control polysomes; (- - - - -), 30 $A_{260}$ units polysomes from LSD-treated animals. Sedimentation is from left to right. The dashed line at the bottom represents a blank gradient. Horizontal bars: A, ribosomal subunits; B, monosomes plus disomes; C, poly-

the following experiments. Postmitochondrial supernatants were prepared from brains of control and LSD-treated animals and fractionated on sucrose gradients into RNP particles, monosomes and polysomes (Fig. 1). The distribution of poly(A+)mRNA in these three fractions was determined by hybridization of [$^3$H]polyuridylate to RNA purified from each fraction. In the controls 2.9% of the mRNA was associated with the monosome fraction while 81.6% was associated with polysomes (Table III). After LSD treatment there was a dramatic increase in the percentage of mRNA associated with the monosomes (from 2.9% to 69.1%) and a decrease in mRNA associated with polysomes (from 81.6% to 23.8%). mRNA did not flow into RNP particles during polysome disaggregation; in fact, somewhat less mRNA was detected in RNP particles after LSD treatment.

To further analyse the monosome complexes, polysomes were purified from the brain of control and LSD-treated animals and fractionated on sucrose gradients into three regions, viz., ribosomal subunits, monosomes plus disomes and polysomes (Fig. 2). The absorbance profile for the polysomes from LSD-treated animals indicated a dramatic increase in monosomes with a concomitant disaggregation of polysomes. The distribution of mRNA in the three fractions was then analyzed by the [$^3$H]polyuridylate hybridization assay. In the control 17.1% of the mRNA was present in the monosome plus disome fraction while 76.8% was associated with polysomes (Table IV). After LSD treatment there is a marked increase in the percentage of mRNA associated with monosome plus disome fraction (from 17.1% to 43.9%) and a decrease in mRNA associated with polysomes (from 76.8 to 44.0%). Since the total mRNA content did not decrease after LSD administration mRNA appeared to shift from polysomes and accumulate on monosomes. As nascent peptides were released normally following LSD [13], the accumulation of mRNA on monosomes could reflect a blockage of protein synthesis at a late initiation or early elongation step.

TABLE III

DISTRIBUTION OF POLYADENYLATED mRNA IN POSTMITOCHONDRIAL EXTRACTS FROM CONTROL AND LSD-TREATED ANIMALS

Postmitochondrial extracts were prepared from three control and two LSD-treated (100 μg/kg for 30 min) animals and layered onto linear 15—40% (w/v) sucrose gradients. Centrifugation was for 4 h at 26 000 rev./min in the SW-27 rotor at 4°C. Gradients were fractionated into three regions (see Fig. 1) and the fractions precipitated overnight with ethanol. RNA in each fraction was purified by phenol extraction and poly(A+)mRNA was measured by hybridization to [$^3$H]polyuridylate [24]. The data is expressed as percent of total radioactivity in each fraction. The mean of two separate experiments is given.

| Fraction | [$^3$H]Polyuridylate bound (cpm × $10^{-3}$) | | Radioactivity (% of fraction) | |
|---|---|---|---|---|
| | Control | LSD | Control | LSD |
| RNP particles | 47.8 | 37.3 | 15.6 | 7.1 |
| Monosomes | 8.8 | 361.2 | 2.9 | 69.1 |
| Polysomes | 250.7 | 124.2 | 81.6 | 23.8 |

TABLE IV

ASSOCIATION OF POLYADENYLATED mRNA WITH RIBOSOMES AND POLYSOMES OF CONTROL AND LSD-TREATED ANIMALS

Polysomes from the control and drug-treated animals were prepared and fractionated as in Fig. 2. RNA from each fraction was purified by phenol extraction and the amount of poly(A+)mRNA determined by the [$^3$H]polyuridylate hybridization assay [24]. The data represent the means of two separate experiments.

| Gradient region | [$^3$H]Polyuridylate bound (cpm) | | Radioactivity (% of fraction) | |
|---|---|---|---|---|
| | Control | LSD | Control | LSD |
| Ribosomal subunits (A) | 2 888 | 2 993 | 6.1 | 12.1 |
| Monosomes plus disomes (B) | 8 575 | 25 120 | 17.1 | 43.9 |
| Polysomes (C) | 30 898 | 20 160 | 76.8 | 44.0 |

*Characterization of the increased monosome pool generated following LSD administration*

Analysis of the monosome peak formed after LSD administration revealed that it was slightly higher in molecular weight compared to the 80S monosome peak in the control animals (Fig. 2). The estimated sedimentation coefficient of this peak was 102S, suggesting the presence of an 80S monosome associated with an average size brain mRNA molecule of 20S. The presence of 80S monosome was confirmed since treatment of the isolated 102S complex with either 0.5 M KCl or 20 mM EDTA revealed the presence of both large and small ribosomal subunits. Dissociability of the complex in 0.5 M KCl suggested the absence of nascent peptides [11].

If mRNA was associated with 80S monosomes as suggested in Table IV, treatment with either high salt or EDTA should release mRNA as RNP particles having a similar sedimentation coefficient to mRNP particles released from polysomes i.e. 20—90S (unpublished data). To investigate this possibility, 102S

TABLE V

DISTRIBUTION OF POLY(A+)mRNA IN LSD-INDUCED mRNA-RIBOSOME COMPLEXES AFTER TREATMENT WITH EDTA AND 0.5 M KCl

The LSD-induced mRNA-ribosome complexes (102S complex) were treated with either 20 mM EDTA or 0.5 M KCl and centrifuged on linear sucrose gradients containing either 20 mM EDTA or 0.5 M KCl. The gradients were fractionated as shown in Fig. 2. RNA was isolated from each fraction by phenol extraction and assayed for poly(A+)mRNA by the [$^3$H]polyuridylate hybridization assay [24]. The percent radioactivity bound is the mean of two separate experiments for each treatment.

| Treatment | Gradient fraction | [$^3$H]Polyuridylate bound | |
|---|---|---|---|
| | | % | cpm |
| EDTA | <40S | 41.2 | 6830 |
| | 40—60S | 30.3 | 5023 |
| | >60S | 28.5 | 4724 |
| 0.5 M KCl | <40S | 40.0 | 8055 |
| | 40—60S | 33.1 | 6675 |
| | >60S | 26.9 | 5410 |

complexes were isolated and centrifuged in the presence of high salt or EDTA gradients. RNA was purified from the three fractions of the gradients shown in Fig. 2 and hybridized to [$^3$H]polyuridylate to locate poly(A+)mRNA (Table V). Treatment with either EDTA or high salt produced similar results, i.e., poly(A+)mRNA was present in mRNP particles in sizes ranging from <40S to >60S. This result provided further evidence that mRNA was present in the 102S complex together with 40S and 60S ribosomal subunits.

*Translation of brain mRNA associated with the blocked 102S complex*

Addition of excess initiation factors has been shown to reverse the initiation block in heme-deprived reticulocytes [30], heat-treated wheat germ extracts [31] and in Ehrlich ascites cells [19,20]. If the LSD-induced inhibition of protein synthesis in brain was the result of the formation of inhibitors of initiation or the initial steps of elongation then the block might be overcome if the 102S complex was translated in a cell-free system containing excess initiation or elongation factors. Blocked 102S initiation complexes were therefore isolated from brains of LSD-treated animals and translated in wheat embryo extracts as described in Materials and Methods. The dose-response curve for the translation of 102S complexes was linear throughout a concentration range of 0.5—1.5 $A_{260}$ units/assay. Translation of an equal amount of brain monosomes plus disomes isolated from control animals (1.5 $A_{260}$ units/assay) produced only a slight stimulation of protein synthesis, i.e., 25% of the value observed for the 102S complex (control, 5100 cpm; LSD, 20 500 cpm). This result further supported that mRNA is present in the 102S complex and that translational factors in the wheat embryo extract can overcome the block in brain protein synthesis which is produced by LSD.

The blocked 102S complex was then examined for the presence of translatable mRNA coding for brain-specific S100 protein. Messenger RNA was isolated from the blocked complex by phenol extraction and translated in the wheat embryo cell-free system. The products of the reaction were assayed for the presence of brain-specific S100 protein by immunoprecipitation with S100 protein antiserum as we have previously described [22,27]. The relative synthesis of S100 protein was 0.36% which is within the range of values (0.27 ± 0.11%) which we have obtained with polysomal poly(A+)mRNA isolated from control animals [22]. This result demonstrated that mRNA in the 102S complex was functionally unaltered since it coded for intact S100 protein polypeptides. The result further indicated that there is no selective effect of LSD on the mRNA coding for S100 protein since the relative synthesis of this protein was similar to that obtained in a reaction programmed with polysomal mRNA isolated from control animals.

## Discussion

In brain a number of diverse treatments lead to polysome disaggregation, including administration of L-3,4-dihydroxyphenylalanine [32], phenylalanine [33], (+)-amphetamine [34] and LSD [11—14], as well as electroconvulsive shock [35], and intracranial hypertension [36]. Little is known about the mechanism of the brain polysome disaggregation; however, in many of these cases it has been suggested that the polysome disaggregation is mediated by a

non-RNAase mechanism and that the decrease in protein synthesis is the result of inhibited reinitiation. In the present study we have investigated the fate of brain mRNA and have shown that brain mRNA is conserved and not degraded after polysome disaggregation and concomitant inhibition of protein synthesis by LSD. Formation of a 102S complex, which contained mRNA and 40S and 60S ribosomal subunits but lacked nascent peptide chains, suggested that LSD inhibited protein synthesis at a specific stage of late initiation or early elongation.

The inhibition of protein synthesis at specific steps in initiation has been demonstrated in response to a variety of environmental conditions. Analysis of the mechanism of inhibition of protein synthesis in heme-deprived reticulocytes [15,16], reticulocyte lysates warmed to 42°C [17], reticulocytes incubated with double-stranded RNA [18], and amino acid-deprived Ehrlich ascites cells [20] has revealed specific lesions at early steps in the initiation process. When reticulocytes are incubated in the absence of heme, a heme-controlled repressor is activated which specifically phosphorylated the 38 000 dalton subunit of eIF-2 [37]. Phosphorylation of eIF-2 prevents the binding of the ternary complex (eIF-2 · GTP · Met-tRNA$_f$) to 40S ribosomal subunits in the presence of other initiation factors [18,37]. Ranu et al. [37] have suggested that phosphorylation of eIF-2 causes a conformational change in the molecule which inhibits the interaction between the ternary complex and ternary complex dissociation factor which is required for formation of the 40S initiation complex [37]. Datta et al. (1978) have recently suggested that heme prevents the formation of the heme-controlled repressor by allosterically blocking the binding of cAMP to the regulatory subunit of the cAMP-dependent protein kinase which converts the proinhibitor (inactive eIF-2 kinase) to inhibitor (active eIF-2 kinase) [38]. Initiation inhibitors with protein kinase activity have also been discovered in Ehrlich ascites cells [19], rat liver [39], *Artemia salina* and wheat germ [40].

In brain, LSD appeared to inhibit protein synthesis by a mechanism which differed from that of the heme-controlled repressor or double-stranded RNA-activated inhibitor in reticulocytes. In reticulocytes an inhibitor is formed which prevents the formation of a 40S initiation complex [15—18]. In brain the block occurred at a later stage in protein synthesis since a 102S complex consisting of mRNA and both 40S and 60S ribosomal subunits was seen to accumulate. The LSD-induced block in brain protein synthesis therefore appeared to occur at either a late stage of initiation or at an early stage of elongation. It is not yet known whether an inhibitor of translation is formed or whether a translational factor is inactivated following LSD administration.

Inhibitors of elongation have been demonstrated in encysted *A. salina* embryos [41], sea urchin oocytes [42], dystrophic mice [43], and in the amphibian during metamorphic regression of the tail muscle [44]. Warner and Huang have shown that encysted *A. salina* embryos contain an elongation inhibitor bound to the 60S subunit of inactive 80S ribosomes [41]. The inhibition of protein synthesis in brain resembled that in *A. salina* in that the block in translation appeared to involve the formation of a blocked 80S complex. A block at this stage of protein synthesis might result from the inability of the complex to react normally with EF-1 · aminoacyl-tRNA or EF-2 to form the

first peptide bond. Alternatively the lesion in protein synthesis might result from conformational changes in either ribosomal proteins or ribosomal-associated proteins contributing to an altered peptidyl-site and improper positioning of Met-tRNA$_f$ into the P-site.

To date the majority of studies investigating translational control mechanisms have been carried out on single cells such as reticulocytes or on cultured cell lines such as HeLa and Ehrlich ascites cells [15—20]. This study on brain protein synthesis is of particular interest since LSD via interaction with specific neurotransmitter receptors elicited a global inhibition of brain protein synthesis in which diverse populations of cell types including neurons and glia were affected [11,12]. Signals must be transmitted intercellularly from one cell type to another, since LSD binds to neuronal receptors; yet synthesis of the glial-specific S100 protein [45] was affected, as mRNA coding for S100 protein shifted from polysomes to monosomes. Since LSD acts initially at the synapse [12], signals must be transmitted intracellularly from the plasma membrane to the translational apparatus. The involvement of membrane receptors was also suggested by the observation that the addition of 1 mM LSD to a brain cell-free protein synthesis system did not inhibit translation (Brown, I.R., unpublished data).

The conservation of mRNA following an inhibition of protein synthesis in liver [46] and Ehrlich ascites cells [47] has indicated that pre-existing mRNA might be used for the recovery phase of protein synthesis. This study demonstrated that brain mRNA was also conserved following an inhibition of protein synthesis, suggesting that pre-existing mRNA might be utilized during polysome reformation. It therefore appeared that some aspects of the regulation of protein synthesis might be similar in a variety of cell types. Although mRNA conservation [46,47] and activation of protein kinases [18,37,38] may be an integral part of translational control in many cells, it is apparent that control of protein synthesis can be regulated at different stages in different cell types. We are currently using a reconstituted cell-free protein synthesis system derived totally from brain to determine the exact location of the LSD-induced lesion of protein synthesis and to investigate whether alterations of ribosomal subunits or initiation or elongation factors are involved in the regulation of brain protein synthesis.

## Note added in proof (Received August 10th, 1979)

Our recent evidence suggests that LSD-induced hyperthermia (i.e. elevation of body temperature) may be involved in the effect of the drug on brain protein synthesis. See Refs. 48—50.

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