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Methionine Sulfoximine *In Vivo* Modifies the $tRNA^{Lys}$ Pool of Developing Rat Brain

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Abstract: tRNA was extracted from brains of 3-, 8-, and 18-day-old rats that were injected intracerebrally, 45 min before death, with [^{3}H]methyl methionine or [8-^{3}H]guanosine, and intraperitoneally, 3 h before death, with L-methionine-*dl*-sulfoximine (MSO), a methylation-activating convulsant agent. Although there was no effect of age or of MSO on the per gram yield of tRNA, its specific radioactivity (dpm/A_{260}) was highest at 3 days in both the control and the MSO groups. Age- and MSO-related changes in the $tRNA^{Lys}$ content of the brain tRNA pool were investigated by means of benzoylated DEAE-cellulose (BDC) and reverse-phase chromatography (RPC). BDC chromatography revealed $tRNA^{Lys}$ species in the brains of the MSO-treated animals that were absent in control brains. Of particular interest was the finding that differences in RPC-5 chromatographic mobility between control and MSO-$tRNA^{Lys}$ species were abolished by conversion to lysyl-tRNA, suggesting that the MSO-elicited change(s) in $tRNA^{Lys}$ structure involved the binding site(s) for lysine. Two additional findings were made: (a) lysine acceptance by the [^{3}H]methyl-labeled $tRNA^{Lys}$ purified from brains of the MSO-treated animals was higher than that of controls at 18 days; and (b) omission of the BDC chromatographic step accentuated the differences in mobility on RPC-5 columns between $tRNA^{Lys}$ species of control and MSO-treated brains. Lastly, we found that some $tRNA^{Lys}$ species present in the MSO-treated brains contained significantly different proportions of N^2-methyl guanine and 1-methyl adenine, relative to controls. These MSO-elicited changes in the methyl base content of $tRNA^{Lys}$ of immature rat brain are the first evidence of an alteration of brain tRNA structure by a centrally acting excitatory agent. **Key Words:** Brain tRNAs—Development—$tRNA^{Lys}$—Methionine sulfoximine—$tRNA^{Lys}$ methylation. **Sellinger O. Z.** Methionine sulfoximine *in vivo* modifies the $tRNA^{Lys}$ pool of developing rat brain. *J. Neurochem.* **38**, 1676–1685 (1982).

Over the past few years, work from our laboratory has shown that immature brain tRNAs are subject to the action of homologous tRNA methyltransferases (Cummins et al., 1975) which convert them into mature tRNA molecules postnatally (Salas et al., 1976; Elahi and Sellinger, 1979), and that this process continues for as long as a month and a half. The analysis of [^{3}H]methyl-tRNAs formed by 3- and 18-day-old rat brain revealed 10 different [^{3}H]-*N*-methyl bases in the newly formed [^{3}H]tRNA (Sellinger et al., 1977; Dainat et al., 1978; Elahi and Sellinger, 1979). More recently, we found that the nature of the [^{3}H]methyl-tRNA base varies in different neural cell types. In the cerebellum of the rat (Dainat and Sellinger, 1980), [^{3}H]-N^2-methyl guanine was the predominant tRNA [^{3}H]methyl base formed throughout the first 43 days of life; in glial astrocytes tested after 19 days in culture

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Abbreviations used: BDC, Benzoylated DEAE-cellulose; DEPC, Diethylpyrocarbonate; EtOH, Ethyl alcohol; MSO, L-Methionine-*dl*-sulfoximine; RPC, Reverse-phase chromatography; RSA, Relative specific activity; SRA, Specific radioactivity.

(Cummins and Glover, 1979) and 1–8 h of exposure to [^{3}H]guanosine, the principal tRNA [^{3}H]methyl guanine was 7-methyl guanine (Sellinger and Der, 1981); whereas in the 3-day-old rat, the newly formed [^{3}H]tRNA contained equal proportions of [7-^{3}H]- and [^{3}H]-N^2-methyl guanine and had no [1-^{3}H]methyl guanine (Sellinger and Der, 1980).

Parallel *in vitro* studies have disclosed that neural tRNA methyltransferases undergo age-dependent changes in specificity (Salas et al., 1976; Dainat and Sellinger, 1980) and that they acquire their adult specificity patterns only gradually, presumably via temporally evolving mechanisms designed to commit them eventually to recognize only their cognate tRNAs. Recently, however, we documented three different instances of "aberrant" interaction between the tRNA methyltransferase protein of neural cells and heterologous tRNA: (a) in the cerebellum of the icteric Gunn rat (Dainat et al., 1979); (b) in the brain of the Sprague-Dawley albino rat following a single dose of the convulsant agent L-methionine-*dl*-sulfoximine (MSO; Salas et al., 1977; Dainat et al., 1978); and (c) in cultured glial astrocytes briefly exposed to MSO (Sellinger and Der, 1981).

In the present report we describe further MSO-elicited modifications in the *in vivo* methylation of immature brain tRNA (Der and Sellinger, 1980) by focusing on the synthesis of an individual tRNA species. $tRNA^{Lys}$ appeared especially suited for this purpose, for its purification (Raba et al., 1979), its numerous, readily separable isoacceptors (Ortwerth et al., 1973; Chu-Der and Ortwerth, 1979), and its content of up to five methyl bases/mole (Raba et al., 1979; Sprinzl et al., 1980) have been described in detail in nonneural systems. In addition, $tRNA^{Lys}$ has been shown to exhibit typical developmental fluctuations in its isoacceptor composition that appear to relate directly to cell division (Conlon-Hollingshead and Ortwerth, 1980), cell differentiation (Kleiman et al., 1978; Lin and Agris, 1980), growth (Chu-Der and Ortwerth, 1979, 1980), and proliferation (Juarez et al., 1975; Marini and Mushinski, 1979).

EXPERIMENTAL PROCEDURES

Animals

The 3-, 8-, and 18-day-old rats were of the Sprague-Dawley strain. The 3-day-old animals were used at the earliest 24 h after arrival from the shippers (Spartan Farms, Haslett, MI). They were housed in cages containing 1 family/cage.

Chemicals

DEAE-cellulose (DE23, Whatman) was from Reeve-Angel, Clifton, NJ. For benzoylated DEAE-cellulose (BDC) chromatography, Cellex-BD (100–200 mesh) was obtained from Bio-Rad, Richmond, CA. For reverse-phase chromatography (RPC), the solid support material RPC-5 was a generous gift of Dr. G. D. Novelli, Oak Ridge, TN. Diethylpyrocarbonate (DEPC) was from Accurate Chemical and Scientific Co., Hicksville, NY; [8-^{3}H]guanosine (15 Ci/mmol) was from New England Nuclear, Boston, MA; L-[U-^{14}C]lysine (270–345 mCi/mmol) and L-[^{3}H]methyl methionine (0.5–1.0 Ci/mmol) were from ICN, Irvine, CA, Amersham Corp., Arlington Heights, IL, or New England Nuclear; and L-[4,5-^{3}H]lysine (75–78 Ci/mmol) was from Amersham or New England Nuclear.

In vivo labeling and extraction of brain tRNA

In these experiments, 4 μCi of [^{3}H]guanosine or [^{3}H]methionine per gram of body weight was injected intracerebrally, and the brains were removed for RNA extraction 45 min thereafter. The cerebral cortices were homogenized in 0.25 *M* sucrose containing 35 m*M* Tris-HCl (pH 7.4), 25 m*M* KCl, 25 m*M* $MgCl_2$, 1 m*M* sodium EDTA, 10 m*M* β-mercaptoethanol, and 0.05% DEPC. The homogenate was centrifuged for 20 min at 30,000 × *g*; the low-molecular-weight RNA was extracted from the resulting supernatant by the procedure of Ortwerth and Chu-Der (1974).

Purification, hydrolysis, and compositional analysis of $tRNA^{Lys}$

Following extraction, the RNA was precipitated with ethanol and stored overnight at −20°C. Following centrifugation, it was redissolved in 10 m*M* Tris-HCl, containing 10 m*M* $MgCl_2$, 1 m*M* EDTA, and 50 m*M* NaCl, pH 7.5 (buffer A), and the sample was loaded on a column of DEAE-cellulose previously equilibrated with buffer A. Elution with buffer A was continued until the A_{260} reading reached a value of 0.05, at which time the tRNA-containing fraction was stripped with 0.7 *M* NaCl in buffer A. The tubes with A_{260}/A_{280} values above 1.5 were pooled and dialyzed to remove NaCl. Following lyophilization, the tRNA was hydrolyzed (Klagsbrun, 1972), and the [^{3}H]guanine and [^{3}H]methyl guanines (after a pulse of [^{3}H]guanosine) or the [^{3}H]methyl bases, including [1-^{3}H]methyl adenine (after a pulse of [^{3}H]methionine), were separated by HPLC according to Salas and Sellinger (1977). Since, however, it was determined in preliminary experiments that brain [^{3}H]$tRNA^{Lys}$ contained no [1-^{3}H]methyl guanine, [^{3}H]guanine was separated from the other [^{3}H]methyl guanines by elution with 50 m*M* ammonium phosphate (pH 3.0) and 0.3 *M* ammonium chloride in 5% (vol/vol) methanol.

BDC chromatography

Cellex-BD was washed and the column (0.7 × 20 cm) was packed and prepared for use according to Roy et al. (1971). The equilibrating buffer was 50 m*M* sodium acetate, pH 5.0, containing 10 m*M* $MgCl_2$ and 0.3 *M* NaCl. tRNA, dissolved in the equilibrating buffer, was loaded, and the elution was carried out with 120 ml of a 0.5–1.0 *M* NaCl gradient in the above buffer. The flow rate was adjusted to 2 ml/min and 5-ml fractions were collected. All solutions used in BDC chromatographic work also contained 0.04% sodium azide. Aliquots (0.1 ml) of each effluent fraction were mixed with scintillant and their radioactivity was determined. Up to 0.5 ml was taken for the aminoacylation assay (see below).

RPC-5 chromatography

The procedure described by Kelmers and Heatherly (1971) was adapted to a 0.9 × 50-cm column which was

operated at approximately 250 psi. Elution was carried out using 200 ml of a gradient of 0.5– or 0.6–1.0 *M* NaCl prepared in 10 m*M* sodium acetate, 10 m*M* $MgCl_2$, 1 m*M* EDTA, and 30 m*M* β-mercaptoethanol, pH 4.5.

Preparation of aminoacyl-tRNA synthetases

Livers of adult rats were homogenized in 2 volumes of buffer containing 0.25 *M* sucrose, 50 m*M* KCl, 10 m*M* Tris-HCl (pH 7.5), 10 m*M* $MgCl_2$, 0.01 m*M* β-mercaptoethanol, and 10% (vol/vol) glycerol. The remainder of the procedure was as described in a previous report (Sellinger and Der, 1980).

tRNA aminoacylation

After chromatography on BDC or RPC-5 (postcharging). tRNA was aminoacylated in a reaction volume of 1 ml containing 1 μCi of [^{14}C]lysine, 0.4 *M* Tris-HCl (pH 7.5), 10 m*M* $MgCl_2$, 20 m*M* KCl, 1 m*M* ATP, 0.6 m*M* CTP, 2 m*M* β-mercaptoethanol, up to 0.5 ml of effluent, and approximately 1 mg of aminoacyl-tRNA synthetase protein. Following incubation for 10 min at 37°C with gentle shaking, the reaction was stopped by placing the tubes on ice, adding 5 ml of ice-cold 10% trichloroacetic acid, and collecting the radioactive lysyl-tRNA on Whatman GF/A filters, processed for counting according to Yang and Novelli (1971).

Before chromatography on RPC-5 (precharging). This was carried out on amounts of tRNA in excess of 1 A_{260} unit in a final volume of 5 ml, containing 5 μCi of [^{14}C]- or [^{3}H]lysine, 0.2 *M* Tris-HCl (pH 7.5), 10 m*M* $MgCl_2$, 20 m*M* KCl, 1 m*M* ATP, 3 m*M* CTP, 8 m*M* β-mercaptoethanol, and 10–15 mg of aminoacyl-tRNA synthetase protein. After incubation at 37°C for 20 min, the incubation mixture was passed through a column of DEAE-cellulose (5-ml bed volume), followed by 50 ml of buffer A, 30 ml of buffer A that was 0.5 *M* in NaCl, and finally, to elute lysyl-tRNA, 40 ml of buffer A that was 0.7 *M* in NaCl. Aliquots of the eluate were removed for counting; 10 A_{260} units of adult liver tRNA was added as carrier and total tRNA was precipitated by the addition of 4 volumes of 100% ethyl alcohol (EtOH). The tRNA was collected after overnight storage at −20°C by filtration over 0.45-μ Millipore filters and was suspended in 1 ml of RPC-5 buffer (see above).

Protein determination

The procedure of Lowry et al. (1951) was used with crystalline bovine serum albumin as standard.

RESULTS

The effect of MSO and age on the yield and the radioactivity of brain [^{3}H]tRNA

[^{3}H]tRNA was prepared from the brains of 3-, 8-, and 18-day-old rats, following a 45-min pulse of [^{3}H]methyl-L-methionine; [8-^{3}H]guanosine was administered to 18-day-old animals as well. The amounts of DEAE-purified, EtOH-precipitated tRNA obtained from widely different initial brain weights are shown in Table 1, together with comparative information on the labeling of this fraction.

The yield of tRNA in the range of 3.0–3.7 A_{260} units/g was not affected by either the MSO treatment or age. Equally similar were the two total radioactivity (dpm × 10^{-5}) values following methionine; following guanosine, however, the total radioactivity of the MSO-[^{3}H]tRNA fraction was about half that of the control [^{3}H]tRNA. Brain tRNA became significantly labeled at all ages, even though in the several experiments carried out, the absolute yield of tRNA ranged from a low of about 10 A_{260} units (8 3-day-old brains) to a high of about 140 A_{260} units (32 18-day-old brains). Additionally, Table 1 shows that following the 45-min pulse, the specific radioactivity (SRA) of [^{3}H]tRNA was highest at 3 days.

Effect of MSO on $tRNA^{Lys}$: BDC chromatography

The identification of $tRNA^{Lys}$ within the tRNA pool of developing rat brain was carried out by BDC chromatography, followed by assay of effluent aliquots for lysine acceptance activity. The BDC profile of $tRNA^{Lys}$ in control and MSO-treated brains at 3, 8, and 18 days (Fig. 1) shows two control species at all three ages, the early eluting species being the major one. An additional $tRNA^{Lys}$ species was detected in the 8- and 18-day MSO profile. The 3-day MSO profile did not show this additional $tRNA^{Lys}$, yet the magnitude of the two existing $tRNA^{Lys}$ peaks was inverted vis à vis the controls, the late eluting peak being the major one in this case.

TABLE 1. *Isolation and labeling of brain tRNA: Effect of postnatal age*

Age (days)	[^{3}H]tRNA precursor	Control				MSO			
		A_{260}	A_{260}/g	dpm (× 10^{-5})	dpm/A_{260}	A_{260}	A_{260}/g	dpm (× 10^{-5})	dpm/A_{260}
3	L-Methionine[a]	9.24	3.61	1.79	16,690	10.1	3.69	1.87	15,310
8	L-Methionine	25.6	2.93	1.81	7060	28.1	3.15	1.26	4485
18	L-Methionine	23.2	3.53	1.50	6450	25.5	3.64	1.57	6150
	Guanosine[b]	142.8	3.28	3.21	2250	132.4	3.26	1.54	1170

The brain weights (g) of the control and MSO-treated rats were 3.0 and 3.1 g at 3 days, 8.7 and 8.9 g at 8 days, and 6.4 and 6.0 g after L-methionine and 43.4 and 40.5 g after guanosine at 18 days.

[a] [^{3}H]Methyl labeled.

[b] [8-^{3}H]Guanosine.

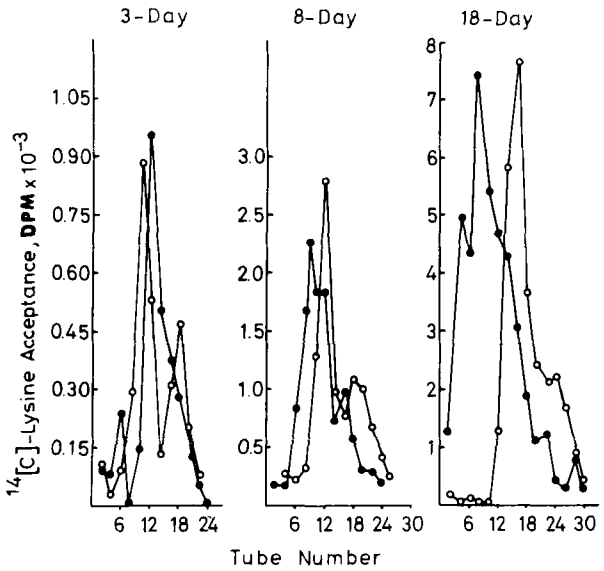

FIG. 1. BDC chromatography of tRNA^{Lys} in brains of 3-, 8-, and 18-day-old rats. For the 3-day-old samples, 9.2 A_{260} units of control (○) and 10.1 A_{260} units of MSO-treated (●) tRNA were placed on a column of BDC (see Experimental Procedures), and the lysine acceptance of every other tube was determined. For the 8-day-old samples, 25.6 and 28.1 A_{260} units were loaded on the column. For the 18-day-old samples, the loaded A_{260} units were 85.3 for the control and 100 A_{260} units for the MSO-treated animals.

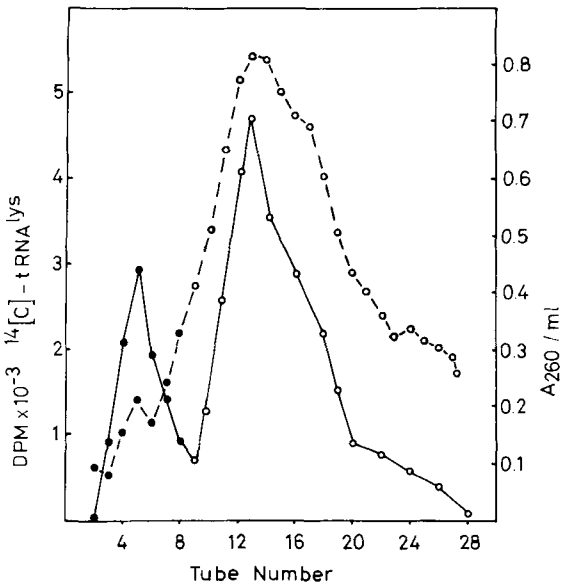

FIG. 2. BDC rechromatography of early eluting MSO-tRNA^{Lys}. "MSO" tubes 2–6 (Fig. 1, 18 days) were pooled with the major tRNA^{Lys} species of control brain (tubes 12–16). Thirty-four control and 11.6 MSO A_{260} units were loaded on a fresh BDC column. Elution was with 80 ml of a 0.3–1.0 *M* NaCl gradient, prepared in the buffer described in Experimental Procedures, and 3.5 ml/tube was collected. (○), Control; (●), MSO treated. Dashed line, A_{260}/ml.

To determine if the additional tRNA^{Lys} found in the 18-day-old MSO brain represents a species absent in the control brain, MSO tubes 2–6 and control tubes 12–18 (Fig. 1) were pooled, the total tRNA was EtOH precipitated, collected, suspended, and rechromatographed. Figure 2 shows the clear resolution of two tRNA^{Lys} peaks, each coincident with a peak of absorption at 260 nm. These findings demonstrate that the administration of MSO resulted in the emergence of a chromatographically distinct tRNA^{Lys} species in the brain of the 18-day-old rat. When the two tRNA^{Lys} species, shown as separate entities in Fig. 2, were first precharged, MSO-tRNA with [^{3}H]lysine and control tRNA with [^{14}C]lysine, and were then rechromatographed as charged species, their resolution could no longer be achieved (Fig. 3). This finding indicates that the structural differences that accounted for their distinct chromatographic behavior in the uncharged form (Fig. 2) were wiped out upon reaction with lysine to form lysyl-tRNA.

RPC-5 chromatography

The major brain tRNA^{Lys} species identified by BDC chromatography were further purified by RPC-5 chromatography (Fig. 4). At 3 days, the control RPC-5 profile of tRNA^{Lys} (tubes 5–13, Fig. 1) displayed a late eluting species that was not detected in the MSO profile (tubes 10–14, Fig. 1). The unique MSO-tRNA^{Lys} at 8 days (tubes 6–10, Fig. 1) gave rise to the complex tRNA^{Lys} profile shown in Fig. 4B whereas the main 8-day tRNA^{Lys} species (control tubes 10–14 and MSO tubes 12 and 13, Fig. 1) resulted in the relatively simple, as well as similar, profiles of Fig. 4C. Figure 4D and E illustrate tRNA^{Lys} in the 18-day-old brain[1] after BDC (D) and after DEAE, omitting BDC (E). It may be seen that differences between the tRNA^{Lys} profiles of control and MSO brains became accentuated in the latter situation.

The labeling of brain tRNA^{Lys}: Effects of age and MSO

Since brain tRNA becomes significantly labeled within 45 min after the intracerebral administration of its precursor, be this [^{3}H]methyl methionine (Saborio and Aleman, 1970; Dainat et al., 1978) or [^{3}H]guanosine (Sellinger and Der, 1980), we used this pulse time in the present study. The SRA of tRNA^{Lys} (dpm/A_{260}) and the effects of the MSO treatment and age on it are shown in Table 2. At 3 days, the two tRNA^{Lys} species clearly resolved by BDC chromatography (in an experiment similar to that resulting in the profile shown in Fig. 1) became markedly, albeit similarly, labeled in the control and

[1] The tRNA^{Lys} was obtained by BDC chromatography in an experiment other than that depicted in Fig. 1 but which was carried out under identical conditions.

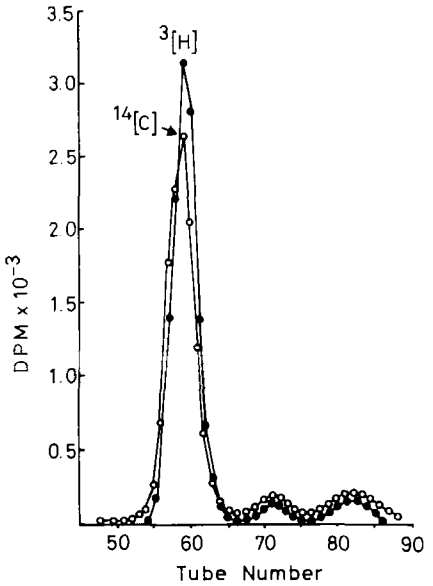

FIG. 3. RPC-5 chromatography of [^{3}H]- + [^{14}C]lysyl-tRNA. The contents of tubes 3–8 and 10–18 (Fig. 2) corresponding, respectively, to 4.21 and 21.5 A_{260} units were EtOH precipitated and stored at −20°C overnight; the precipitated tRNA^{Lys} was collected on 0.45-μ Millipore filters. After suspension in water and determination of the absorption at 260 nm, equal amounts of tRNA (2.42 A_{260} units) were charged with lysine (see Experimental Procedures). The control tRNA^{Lys} (tubes 10–18, ○) was charged with [^{14}C]lysine, and the MSO-tRNA^{Lys} (tubes 3–8, ●) was charged with [^{3}H]lysine. Following incubation at 37°C for 20 min, each sample was passed through a 5-ml (bed volume) column of DEAE-cellulose which was eluted sequentially as described in Experimental Procedures. To each of the two resulting 0.7 *M* NaCl fractions (40 ml) were added 10 A_{260} units of adult rat liver tRNA and 160 ml of absolute ethanol (ice-cold), and the total tRNA was allowed to precipitate overnight at −20°C. It was collected on filters, as above, and suspended in 0.5 ml of RPC-5 buffer (see Experimental Procedures). The two samples (17,000 dpm of [^{14}C] and 28,280 dpm of [^{3}H]) were then mixed and chromatographed.

MSO-treated brains, with the minor component (tubes 16–20, −21) exhibiting twice the SRA of the major tRNA^{Lys} species (tubes 8–14). At 8 days, the two principal tRNA^{Lys} species, common to both the control and the MSO-treated brains, became labeled to an approximately equal extent, yet the control species reached significantly higher SRA values. The tRNA^{Lys} species detected only in the MSO-treated brains (BDC tubes 6–10, Fig. 1) was the least heavily labeled, yet, as shown by the dashed line profile of Fig. 4B, its further purification by RPC-5 chromatography gave rise to a radioactivity profile that matched the tRNA^{Lys} acceptance activity quite reasonably. At 18 days, following [^{3}H]methyl methionine (Table 2A), the initial DEAE fraction as well as the two main tRNA^{Lys} species contained in the RPC-5 tubes, as indicated, became labeled to a similar extent. When [^{3}H]guanosine was used (Table 2B), the SRA values of the purified tRNA^{Lys} species were significantly lower than after [^{3}H]methyl methionine.

The effect of MSO on the acceptance capacity of brain tRNA^{Lys}

A series of preliminary experiments was conducted in which differences in lysine acceptance capacity between the brain tRNA of control and MSO-treated rats were sought. In these experiments, we compared: (a) the initial acceptance rates (1–5 min) of 0.4 A_{260} units of tRNA (after DEAE-cellulose, see Experimental Procedures), using 0.05 and 0.1 mg of liver aminoacyl-tRNA synthetase protein; (b) the time course of lysine acceptance (5–45 min) in the presence of 0.4 A_{260} units of tRNA and at aminoacyl-tRNA synthetase levels ranging from 0.05–1.5 mg protein; (c) the time course of lysine acceptance (5–45 min) in the presence of 1 mg of aminoacyl-tRNA synthetase protein and at levels of tRNA ranging from 0.1–1.5 A_{260} units/tube; and (d) lysine acceptance at 0.1–3.0 A_{260} units of tRNA in the presence of excess (1 mg protein) aminoacyl-tRNA synthetase and with incubation for 5 and 45 min. No consistent differences in lysine acceptance were uncovered between the relatively crude "bulk" brain tRNA preparations isolated, respectively, from 3-day-old control and MSO-treated animals (see, however, Der and Sellinger, 1980). However, we consistently noted a higher acceptance activity for the "MSO species" using 18-day-old, RPC-5-purified tRNA^{Lys} (Table 3), particularly after [^{3}H]methionine.

The methylation of brain tRNA^{Lys}: Effects of MSO

The principal tRNA^{Lys} species were hydrolyzed (Figs. 1 and 4), their [^{3}H]methyl bases were separated by HPLC, and their radioactivity was determined. The three major [^{3}H]methyl bases found present in all hydrolysates of brain tRNA^{Lys} were 1-methyl adenine, 7-methyl guanine, and N^2-methyl guanine, a finding in accordance with the known sequences of mammalian tRNA^{Lys} (Sprinzl et al., 1980). Their sum was normalized to 100%, and the percent of each in the different tRNA^{Lys} species isolated from control and MSO-treated brains was calculated (Table 4). Supplementary findings were expressed as ratios of [^{3}H]methyl bases, with two of them demonstrating effects of MSO on tRNA^{Lys} methylation. Four instances of such effects were encountered: (a) a virtual doubling of the N^2-methyl guanine to 1-methyl adenine ratio in the main MSO-tRNA^{Lys} species at 3 days, (b) a virtual doubling of the N^2-methyl guanine to 7-methyl guanine ratio in the predominant MSO-tRNA^{Lys} at 8 days, coupled to (c) a corresponding 90% reduction of the N^2-methyl guanine to 1-methyl adenine ratio, and (d) a 50% increase in the N^2-methyl guanine to 1-methyl adenine ratio in the early eluting MSO-tRNA^{Lys} species (Fig. 4E) at 18 days.

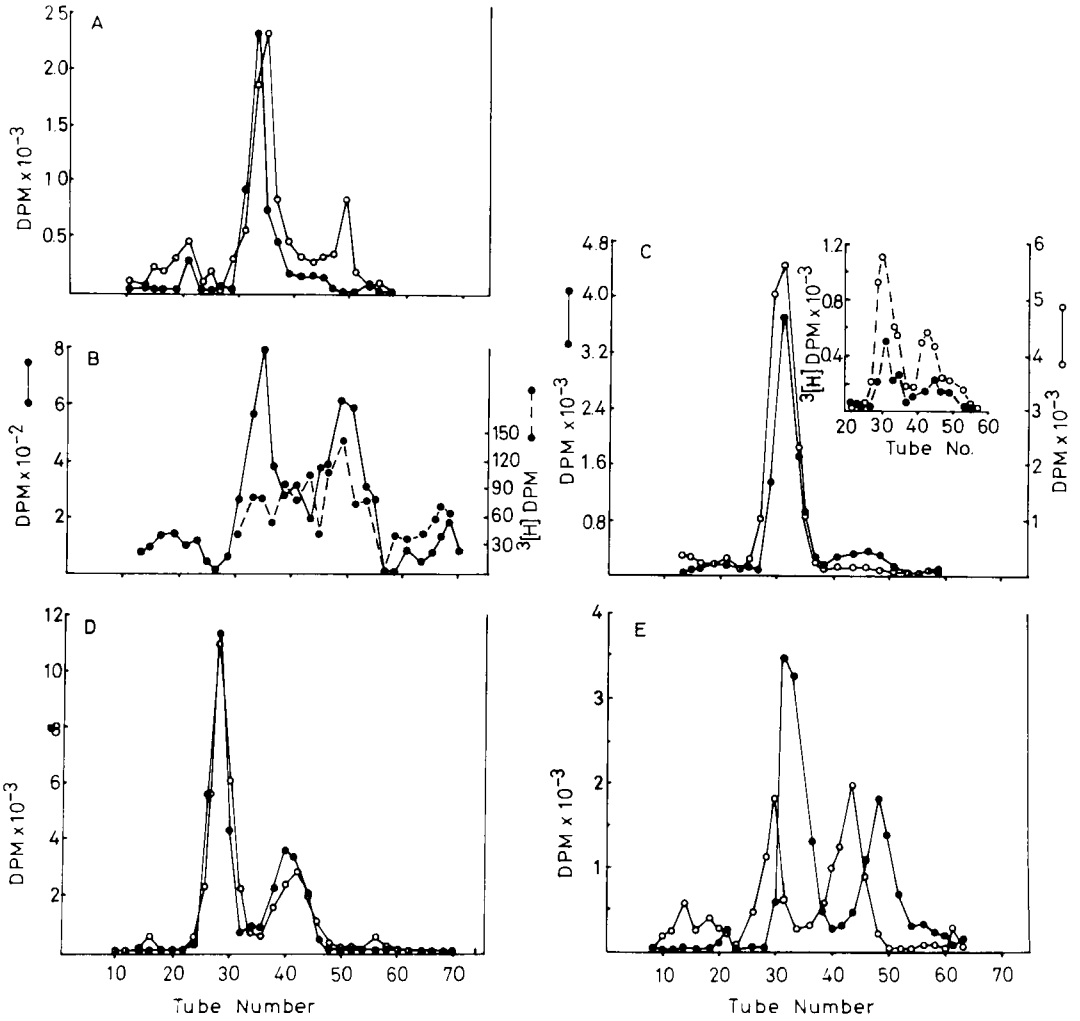

FIG. 4. RPC-5 chromatography of brain tRNA^{Lys} from 3-, 8-, and 18-day-old control and MSO-treated rats. **A** refers to 3-day-old animals, **B** and **C** (with inset) to 8-day-old animals, and **D** and **E** to 18-day-old animals. (○), Control samples; (●), MSO-treated samples. Solid line, [^{14}C]Lysine acceptance activity; dashed line, [^{3}H]-radioactivity, following incorporation of [^{3}H]methyl methionine into the [^{3}H]tRNA methyl bases. **A:** Tubes 8–13 (control) and 10–14 (MSO) of the 3-day BDC run (Fig. 1) were pooled, and their tRNA^{Lys} composition determined in two separate and consecutive chromatographic runs. The A_{260} units loaded onto the RPC-5 columns were 2.7 and 2.9 for the control and the MSO samples, respectively. **B:** The contents of tubes 6–10, 4.0 A_{260} units and 13,572 [^{3}H] dpm, (Fig. 2, MSO, 8 days) were chromatographed. Solid line, [^{14}C]Lysine acceptance; dashed line, [^{3}H]-radioactivity. **C:** As in B, except that the contents of tubes 10–14 (control) and 12 and 13 (MSO) of Fig. 1 (8 days) were chromatographed. Inset shows the [^{3}H]-radioactivity profile, following labeling of the animals with [^{3}H]methyl methionine. Note different ordinate scales for control and MSO samples; 11.7 (control) and 7.2 (MSO) A_{260} units, containing 72,468 and 31,176 dpm of [^{3}H] were loaded on each of two identical columns. **D:** The principal tRNA^{Lys} peak, obtained by BDC chromatography in an experiment analogous to that shown in Fig. 1 (18 days), was chromatographed. 39.8 and 52.1 A_{260} units of control and MSO material were loaded, and the acceptance of [^{14}C]lysine was determined in the eluates. **E:** tRNA^{Lys} was chromatographed immediately following the DEAE-cellulose purification step, omitting BDC chromatography. 11.6 (control) and 12.7 (MSO) A_{260} units were loaded onto each of two identical columns.

DISCUSSION

In the present study, we continue to characterize the changes in the tRNA pool of rat brain during early postnatal development, a time when the many tRNA-processing and -modifying enzymes, actively at work (Elahi and Sellinger, 1979), are particularly vulnerable to a variety of environmental influences (Cummins et al., 1975; Salas et al., 1976, 1977; Sellinger et al., 1977; Dainat et al., 1978, 1979; Sellinger and Der, 1981). Our results regarding the tRNA content of rat brain (Table 1) in the 3- to 18-day postnatal period show values of approximately 0.15 mg/g (1 A_{260} unit = 45 μg of tRNA), matching those previously reported by Harris and Maas (1974). Table 1 also shows that within the range of brain weights tested (3–43 g), the yield of tRNA was relatively independent of their initial value.

The BDC profiles of tRNA^{Lys} (Fig. 1) are the first, to our knowledge, of individual tRNA species in the

TABLE 2. *The effect of MSO on the labeling of tRNA^{Lys} in 3-, 8-, and 18-day-old rat brain*[a]

Age (days)		Treatment		dpm A_{260}	% of Control
3		Control	BDC 8–13[b]	29,800	
		MSO	BDC 10–14	24,100	81
		Control	BDC 16–20	53,200	
		MSO	BDC 16–21	48,600	91.2
8		MSO	BDC 6–10	3390	[c]
		Control	BDC 10–14	6195	
		MSO	BDC 12–13	4390	70
		Control	BDC 17–21	5530	
		MSO	BDC 15–19	3525	63.5
18	(A)	Control	after DEAE	6452	
		MSO	after DEAE	6154	95.5
		Control	RPC 26–32	5380	
		MSO	RPC 31–37	5036	93
		Control	RPC 40–47	8422	
		MSO	RPC 45–51	7844	93.5
	(B)[d]	Control	after DEAE	2248	
		MSO	after DEAE	1166	51.5
		Control	RPC 25–32	2788	
		MSO	RPC 25–31	1104	39.5
		Control	RPC 38–46	3064	
		MSO	RPC 38–45	1324	43.2

[a] [^{3}H]Methyl methionine was injected.
[b] Numerals refer to range of tubes containing the species tested.
[c] Control peak not detected.
[d] [^{3}H]Guanosine was injected.

developing rat brain. Previously, tRNA isoacceptor species have been examined by RPC chromatography in adult calf (Murthy et al. 1974; Borkowski and Brzuskiewicz-Zarnowska, 1975), goldfish (Kaplan and Sirlin, 1975), and mouse brain (Frazer and Yang, 1972; Marini and Mushinski, 1979). Only Frazer and Yang (1972) and Marini and Mushinski (1979) have reported more than one tRNA^{Lys} species.

TABLE 3. *The effect of MSO on the lysine acceptance of brain tRNA^{Lys}*[a]

tRNA precursor		Lysine acceptance: dpm A_{260}	% of Control
A. [^{3}H]Methyl methionine			
Control	RPC 26–32[b]	14,710	
MSO	RPC 31–37	28,785	202
Control	RPC 40–47	16,550	
MSO	RPC 43–51	25,050	151
B. [^{3}H]Guanosine			
Control	RPC 25–32	14,964	
MSO	RPC 29–36	19,070	127
Control	RPC 38–46	10,702	
MSO	RPC 37–44	9965	93

For experiment A, see RPC-5 profile of Fig. 4E; for experiment B, the RPC-5 profile closely resembled that shown in Fig. 4D. A and B of this table are the same as A and B of Table 2.
[a] 18-day-old rats were used.
[b] Numerals refer to the RPC-5 effluent tubes containing the tRNA species tested for [^{14}C]lysine acceptance.

The use of BDC rather than RPC-5 chromatography for the initial survey of the tRNA^{Lys}-isoaccepting species of developing rat brain and for the detection of MSO-elicited changes in its structure (Fig. 1) was prompted mainly by the reported inability of RPC-5 chromatography to reveal any major structural alterations between tRNA from normal and ethionine-treated rats (Ginzburg et al., 1979). A further incentive to use BDC was the high discriminatory power of this technique for revealing differences between uncharged and charged tRNA-isoaccepting species, which allowed Meza et al. (1977) to detect a tRNA^{Ala} isoacceptor under conditions where the analysis of charged species missed it. As may be noted by inspection of Fig. 1, the choice of BDC over RPC-5 proved fortunate, since it led to the discovery of uncharged tRNA^{Lys} species emerging after MSO and not detected among the control tRNAs (Figs. 2 and 3).

The loss of the difference in mobility on BDC columns between tRNA^{Lys}-isoaccepting species of control and MSO-treated animals that occurred upon lysyl-tRNA formation (Fig. 3) is at present difficult to interpret. Yet, obviously, this result implies that the MSO-elicited modification(s) in tRNA^{Lys} structure does not contribute to define the

TABLE 4. *The effect of MSO on the methylation of brain $tRNA^{Lys}$*

Age (days)	Treatment	1-mA (%)	7-mG (%)	N^2-mG (%)	N^2-mG / 7-mG	MSO / Control	N^2-mG / 1-mA	MSO / Control
3	C—BDC 8–13 RPC 32–37	41.7	17.7	40.6	2.30		0.97	
	M—BDC 10–14 RPC 30–35	26.3	23.7	50.0	2.10		1.90	
8	M—BDC 6–10[a] RPC 31–39	43.0	11.7	45.3	3.76	—	1.05	—
	RPC 47–55	33.2	21.3	45.5	2.11	—	1.37	—
	C—BDC 10–14 RPC 27–36	38.0	26.5	35.5	1.34		0.93	
						1.95		0.54
	M—BDC 12–13 RPC 27–35	59.0	11.3	29.7	2.62		0.50	
18	C—RPC 26–32[b]	38.4	27.7	33.9	1.22		0.88	
						1.24		1.52
	M—RPC 31–37	31.0	27.5	41.9	1.51		1.33	
	C—RPC 40–46	43.5	26.4	30.1	1.14		0.69	
						1.08		0.88
	M—RPC 45–52	47.4	23.5	29.1	1.24		0.61	

1-mA, 1-Methyl adenine; 7-mG, 7-methyl guanine; N^2-mG, N^2-methyl guanine; C, control; M, MSO.
[a] There was no early eluting C peak (see Fig. 1, 8 days).
[b] RPC-5 chromatography followed the DEAE purification step; BDC chromatography was omitted (see Fig. 4E).

mobility of lysyl-tRNA; hence it (they) must reside at or close to the many sites that are apparently important for synthetase-tRNA interactions (Schimmel and Soll, 1979) and that become occupied when this interaction is completed. The characterization of the $tRNA^{Lys}$ site(s) modified by MSO must await the rigorous purification of the $tRNA^{Lys}$ species involved (Raba et al., 1979) and the comparison of its (their) interaction *in vitro* (relative to that of control $tRNA^{Lys}$ species) with the purified rat liver lysyl-tRNA synthetase recently dissociated successfully from the 24S aminoacyl-tRNA synthetase complex containing the lysyl-, leucyl-, and arginyl-tRNA synthetase activities (Johnson et al., 1980). RPC-5 chromatography of the major BDC-$tRNA^{Lys}$ species failed to enhance the separation of control species from any species putatively modified by MSO; yet, as shown in Fig. 4E, a clear discrimination between control and MSO-$tRNA^{Lys}$ species could be achieved by omitting BDC chromatography and applying RPC-5 chromatography immediately after the DEAE-cellulose purification step.

The effects of MSO on the labeling of $tRNA^{Lys}$ and on its functional capacity revealed a marked increase in the apparent acceptance capacity of [^{3}H]$tRNA^{Lys}$ after [^{3}H]methyl methionine, whereas after [^{3}H]guanosine, its acceptance capacity remained relatively unchanged. An increase in the acceptance capacity of rat liver $tRNA^{Met}$ was recently stated to be the result of the formation of "active intermediates (which) apparently modify tRNA and thus enhance its acceptance for methionine" (Hradec et al., 1979). Ginzburg et al. (1979) described changes in rat liver tRNA acceptance following the administration of ethionine; these included a decreased capacity for phenylalanine, an increased capacity for histidine, and no change in acceptance for leucine. Three putative causes of these changes were enumerated: ethylation of tRNA, its undermethylation-altered processing, and/or changes in its conformation. As shown in Table 4, the major MSO-$tRNA^{Lys}$ species in the 18-day-old brain (Fig. 4E, Table 3A) had twice the lysine acceptance activity of the corresponding control species, a 52% increase in its N^2-methyl guanine to 1-methyl adenine ratio, and an increased N^2-methyl guanine content (from 33.9–41.9%). Yet, as Table 3 also shows, the acceptance activity of the minor MSO-$tRNA^{Lys}$ species increased by 51%, despite no change (Table 4) in its N^2-methyl guanine to 7-methyl guanine and 1-methyl adenine ratios. A similar, but less pronounced, increase in acceptance by the major MSO-$tRNA^{Lys}$ (Table 3B) was also seen following [^{3}H]guanosine. These observations suggest that the magnitude of the change in lysine acceptance is dependent on changes in the methylation of guanine and adenine (Salas and Sellinger, 1978), and not of guanine alone. The present results give further credence to our previous claim that MSO stimulates a set of N^2-guanine-tRNA methyltransferases (Salas et al., 1977; Dainat et al., 1978; Sellinger and Der, 1981) to insert a methyl group(s) into specialized subpopulations of $tRNA^{Lys}$ isoacceptors (Hatfield et al., 1980; Hedgcoth et al., 1980)

and/or their immature precursors (Elahi and Sellinger, 1979).

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