Brain Ribosomal RNA Synthesis Inhibited by Chlorpromazine (37286)

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(Introduced by K. Stern)
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The influence of chlorpromazine (CPZ) on cerebral turnover of catecholamines has been well established (1–3). Evidence that a specific increase in tyrosine–dopamine transfer is caused by the drug has also been reported (4, 5). Recently a protein–chlorpromazine complex has been demonstrated and an effect of the CPZ-free radicals on ATPase has been shown (6, 7). Previous studies have indicated that CPZ inhibits brain mitochondrial oxidative phosphorylation, acting as an uncoupler (8) and inhibiting oxidation of tricarboxylic acid cycle substrate (9). The drug inhibits orotic acid incorporation into nucleic acids in *Bacillus megaterium,* but it does not affect incorporation of adenine or thymidine. The present study deals with the effect of CPZ on *in vivo* RNA synthesis in rat brain.

Materials and Methods. Male, Charles River rats, weighing 200–300 g were used. Plastic cannulas (Yeda, Rehovot, Israel. Cat. No. 92-103) were implanted 2.5 mm lateral and 2.0 mm posterior to the bregma. The rats were paired at random into controls and experimental animals. Seven days after cannulation, 2 mg CPZ in 0.5 ml were injected ip twice a day during 10 days. Control rats were also injected ip with the same volume of saline. The rats were kept at 20–25° and fed *ad libitum.*

After CPZ treatment the animals were superficially anesthetized with ether and 50 μCi of ^{3}H-uridine (15 Ci/mmole, Israel Nuclear Research Center) in 5 μl saline were injected with an 11 mm needle into the lateral ventricle through the cannula with a microsyringe.

Eighteen hours later the rats were decapitated. The brain was homogenized in TMKB buffer (Tris–HCl buffer 0.05 *M* (pH 7.8), 0.01 *M* MgAc; 0.5 *M* KCl Bentonite 2%). All manipulations were done at 4°. The amount of radioactive precursor in the brain was quantitated by counting 0.1 ml of each brain homogenate as described below.

The ribosomal fraction was isolated by procedure (B), as described by Murthy and Rappaport (11). The ribosomal RNA was extracted by adding sodium lauryl sulfate to a final concentration of 0.5% to the ribosomal fraction resuspended in Tris–HCl buffer (pH 7.4; 0.001 *M*). After 2–5 min, an equal volume of 70% cold phenol was added, and the mixture was shaken for 60 min at 5°. The mixture was centrifuged at 15,000*g* for 10 min; 2.5 vol of cold ethanol were added to the upper layer and kept at —18° for at least 1 hr. Nucleic acids were precipitated by centrifugation (15,000*g*, 10 min) and the precipitate was washed twice with 70% cold ethanol. RNA was extracted from the supernatant fraction after high speed centrifugation. The soluble fraction was diluted with 2 vol of Tris–HCl buffer (pH 7.4; 0.01 *M*) and treated by sodium lauryl sulfate–phenol–ethanol as described for rRNA extraction. The radioactivity and the OD 260 nm were estimated and the specific radioactivity was calculated.

Sucrose gradients of 4 to 20% were prepared in 5 ml tubes in Tris–HCl buffer (pH 7.6; 0.05 *M*).

The gradients were run for 4 hr at 4° at 40,000 rpm in a S.W. 50 rotor of an L-2 Spinco centrifuge.

The tubes were punctured at the bottom and two drop fractions were collected. To each fraction 1 ml H_2O was added and the

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TABLE I. The Effect of Chlorpromazine on Body Weight of Experimental Rats.[a]

Rat no.	CPZ experimentals (g)		Saline controls (g)	
	Start	Finish	Start	Finish
1	215	209	215	220
2	230	235	230	240
3	215	220	220	245
4	230	235	230	250
5	220	225	220	235
6	215	210	215	230
Mean	221	222	221	253

[a] Twelve, male Charles River rats, weighing about 220 g had plastic cannulas implanted as described under Materials and Methods. The rats were paired at random into two groups of six animals each: controls and experimental animals. The rats were kept at 20–25° and fed *ad libitum*.

amount of RNA was estimated at 260 nm in a Beckman spectrophotometer. The fractions were examined for radioactivity in Bray's (12) solution in a Packard Tri-Carb scintillation counter. The results were corrected for yield and internal quenching.

Results and Discussion. In order to determine the effect of the chlorpromazine treatment on the experimental animals, the food intake and weight change of both experimental and control rats was measured. The food actually consumed by the chlorpromazine treated rats was 120 g each while the control rats ate 150 g each.

As shown in Table I, the chlorpromazine-treated rats did not lose weight in spite of eating less than the control animals. Thus the effects of chlorpromazine on brain cell RNA synthesis presented cannot be ascribed to malnutrition or weight loss.

The distribution of injected material into the brain has been established by the injection of methylene blue solution through the implanted cannula with an 11 mm needle into the ventricle.

After the injection the brain was removed and the distribution of the injected dye was determined. By this method it could be established that the injected dye did indeed penetrate into the lateral ventricle. Based upon this observation, the described method of application of material was used for the administration of ^{3}H-uridine, with the aim of following its incorporation into RNA.

Preliminary experiments (Fig. 1) showed that after 18 hr incorporation of radioactive uridine into ribosomal RNA reached a level suitable for evaluation of our experiments. Therefore, all experiments were done at this interval following injection of radioactive uridine.

Six experiments were done in each of which

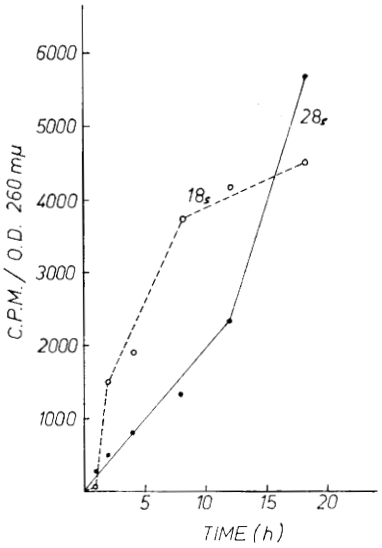

FIG. 1. Incorporation of ^{3}H-uridine in brain ribosomal RNA at different times after injection. Fifty micro-curies of ^{3}H-uridine were injected into the lateral ventricle of 6 pairs of Charles River rats. After the indicated times the brain ribosomal RNA of one pair was extracted and run on sucrose gradients as described. The specific radioactivity at the 28s and 18s peaks obtained on the gradients were calculated and plotted versus time.

TABLE II. ^{3}H-Uridine Incorporation in Brain Ribosomal RNA in Chlorpromazine and Saline-Treated Rats.

Expt no.	Total cpm in brain homogenate ($\times 10^6$)		cpm ^{3}H-Uridine/OD 260 nm			
			28s RNA		18s RNA	
	Control	CPZ-treated	Control	CPZ-treated	Control	CPZ-treated
1	1.65	1.60	7300	7100	8100	8000
	1.52	1.55	4300	3800	8000	4400
2	1.37	1.40	3540	4800	6200	5900
	1.38	1.38	5800	3900	7900	5900
	1.35	1.37	5200	3520	6300	5250
3	0.95	0.97	2860	730	4400	1200
	1.05	1.02	2620	1960	4000	2400
4	1.83	1.75	5600	5240	1000	7800
	1.70	1.75	7650	6400	11,000	9100
	1.45	1.46	7200	6000	9700	7500
	1.65	1.62	6700	4000	9300	7100
5	1.05	1.10	4210	2320	4550	2400
	1.06	1.06	2800	1930	2720	2120
6	1.04	1.02	3100	1300	4600	1400
	0.95	1.00	2300	1380	2700	1700
	1.40	1.40	9800	1920	8200	2500
	1.20	1.17	4100	1860	4700	2400

2 to 4 pairs of rats were used.

In all examined pairs, the specific activity of the radioactive material was higher in the controls compared with the CPZ-treated rats (Table II).

Figure 2 shows a typical gradient profile of the brain ribosomal RNA extracted from rats injected with CPZ or saline. The specific activities at the peak fraction ranged from 1200 to 11,000 cpm/OD 260 nm with all experiments showing incorporation about 30% higher in control than in CPZ-treated rats. Statistical analysis showed a confidence interval [Spiegel (13)] of 280–3800 cpm for $p = 0.05$ at the 28s RNA peak and 120–2580 cpm at the 18s RNA peak (Table II). For estimation of the s values the data of Compagnoni *et al.* (14) were used.

The data presented show that injection of CPZ to rats causes a decrease in ribosomal RNA synthesis in the brain. This phenomenon could be explained by lack of an energy source, such as ATP. The influence of CPZ on ATP metabolism was studied by Akera and Brody (7) and Abood (8). According to these reports, the drug inhibits ATP formation by oxidative phosphorylation in brain mitochondria. A decrease in ATP may severely depress RNA synthesis.

In our experiments, significant differences were found only in the ribosomal RNA, in both 18s and 28s fractions, but no significant changes were observed in the synthesis of soluble RNA extracted from the supernatant as obtained after high speed centrifugation for precipitation of the ribosomes. This indicates that the inhibitory effect of the drug is more specific and might involve the DNA ribosomal RNA transcription site. Nevertheless, the possibility that the discrepancy between the radioactive incorporation into ribosomal RNA and soluble RNA is influenced by their different turnover capacities cannot be excluded.

Summary. Injection of chlorpromazine in Charles River rats causes a depletion in ^{3}H-uridine incorporation into the brain ribosomal RNA. This phenomenon occurs in both 18s and 28s fractions.

An effect on transcription may explain the

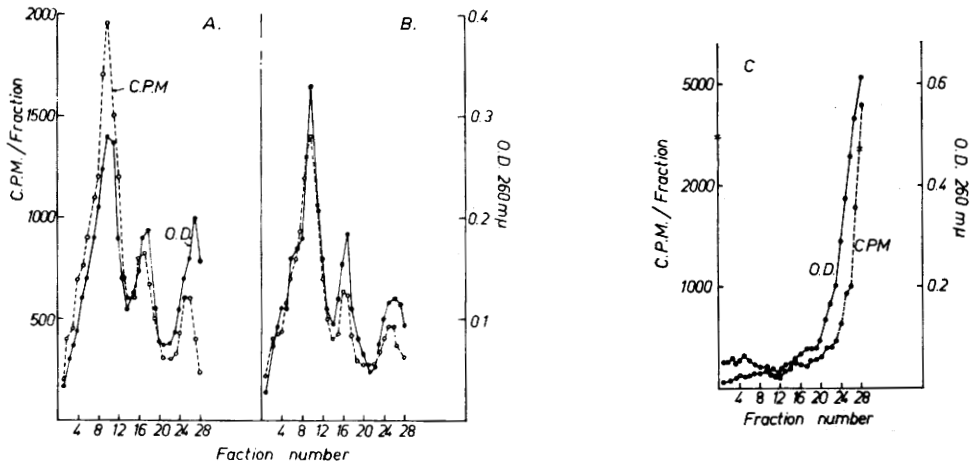

FIG. 2. Sucrose gradient profile of brain ribosomal RNA. ^{3}H-Uridine labeled ribosomal RNA extracted from brains of saline injected rats (control) (A) and from CPZ-treated rats (B) were run on a 4–20% sucrose gradient for 4 hr at 40,000 rpm. Two drop fractions were collected from the bottom. (C) RNAse treated rRNA (20 μg/ml, 30 min, 37°). After 1 ml H_2O was added, the OD 260 nm and radioactivity were determined in each fraction.

reported influences of the drug on brain metabolism.

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