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Base Composition of Ribosomal Ribonucleic Acid in Newborn and Adult Rat Brain. (31040)

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The chemical changes that occur during organ ontogeny may be assumed to reflect the altering metabolic requirements that are a concomitant of morphological development. The recent efforts of a number of laboratories to establish a molecular basis of memory and learning would indicate that chemical changes may also occur as a result of experiential stimuli, and ribonucleic acid (RNA) has been suggested as participating in this process(1-7). In the adult brain if any RNA changes occur after maturation they may be related to some adaptive or mental process, whereas in the newborn animal structural development might also involve changes in RNA. However, it would appear that if no differences in RNA composition could be demonstrated between the young and adult brain the concept of RNA base alterations as a chemical mediator in information processing might be in question.

Hydén has proposed that memory is coded in RNA in a manner analogous to the genetic coding of chromosomal DNA(8). If development and/or learning alters RNA base composition, it should be possible to demonstrate that newborn rat brain, with a minimum of input, is different from the brain of adult animals in this regard. It would not be possible to differentiate between changes due to structural and psychological development, but if no differences were apparent, change in base composition as a mediator in development and learning would necessarily be predicated on equal and opposite changes occurring for these two variables. Another possibility for the lack of any differences resides in localized brain areas undergoing base composition alterations which are opposite and equal irrespective of the nature of the stimulus.

This study is designed to ascertain whether differences in ribosomal-RNA base composition exist between neo-natal and maturated rat brain, as a first approach to the problem of RNA mediating memory and learning processes in brain.

Methods. Newborn rats were obtained as soon as possible post-partum, and at the time of decapitation and removal of the brains were 1-18 hours of age. In some cases the mothers of the litters were used as adult experimental animals. Since the sex of the newborn was not determined, this was deemed desirable so that any sex differences between the two groups could be negated.

The brains of approximately 30-40 newborn rats and 3-5 adult brains were pooled for each analysis. The brains were homogenized in 0.32 M sucrose, .001 M $MgCl_2$, .0004 M KH_2PO_4, .0004 M K_2HPO_4, pH 6.8. The debris, nuclear and mitochondrial fractions were removed by centrifugation in a Spinco model L ultracentrifuge at 18,000 $\times$ *g* for 8 minutes and decantation of the supernatant. The sediment was then washed, recentrifuged; the wash was added to the first supernatant. This was then spun at 90,000 $\times$ *g* for 75 minutes in a #40 rotor. The microsomal pellet obtained was extracted by the method of Kirby(9) as modified by Ding-

TABLE I. Ribosomal RNA and Its Base Composition in Neo-Natal and Adult Rat Brain.

	Wt of brain, mg*	RNA (O.D. 260 mμ) g wet brain	RNA (O.D. 260 mμ) Total brain	% Molar distribution UMP	GMP	CMP	AMP	Pur./Pyr.	G+C/A+U
Neo-natal	240	25.9	6.3	20.2	34.5	27.7	17.5	1.10	1.65
Adult	1550	12.0	18.5	19.7	32.9	27.4	20.0	1.12	1.52
S.D.				±.62	±1.07	±1.01	±.98	±.04	±.06
t				1.68	3.40	.80	5.59	1.10	4.83
P value				.10	<.01	.42	<.01	.30	<.01

* Weight of the brain was determined by dividing the total weight of each pool by the number of brains in the pool.

man and Sporn(10). The pellet was homogenized with 10 ml of .05% sodium lauryl sulfate, stirred for 10 minutes; an equal volume of phenol was added and the mixture then stirred for 60 minutes. The aqueous layer and the protein precipitate were then removed and washed by stirring for 30 minutes with ½ volume of phenol and then centrifuged at 10,000 × *g* for 30 minutes using a SW 25.1 rotor. This extraction step was repeated and the aqueous layer was removed and adjusted to 9 ml with H_2O. One ml of 20% $KC_2H_3O_2$ was added, the solution shaken, and 2-2.5 vol of 95% ethanol was added. The sample was left to stand overnight at 2°C. The ribosomes were collected after centrifugation, redissolved in 5 ml of 2% $KC_2H_3O_2$, reprecipitated with 95% ethanol, and repurified once more. The RNA recovered was placed in a vacuum desiccator and dried over P_2O_5.

The dry RNA was hydrolyzed by alkaline hydrolysis with 0.3 N KOH for 18 hours at 37°C. The hydrolysate was placed in an ice-bath and neutralized with cold 6 N $HClO_4$ with stirring, and the supernatant containing the nucleotides was recovered by centrifugation.

Analysis for base composition was performed as described by Katz and Comb(11). An aliquot of the hydrolysate containing 10-15 O.D. units (260 mμ) was adjusted to 0.05 N HCl and placed on a Dowex 50 × 4 column and eluted with 6 ml 0.05 N HCl and 7.5 ml of H_2O to recover UMP and GMP respectively. The remaining nucleotides were eluted with 40-50 ml H_2O and the effluent placed on a Dowex 1 × 8 column. CMP and AMP were then eluted with 70 ml 0.05 N HCOOH and 70 ml of 1.0 N HCOOH respectively in 10 ml aliquots.

For calculations of the molar distribution of the nucleotides the extinction coefficients as determined by Katz and Comb were used (11). The optical density at 260 mμ of the hydrolysate adjusted to 0.05 N HCl was obtained for determining relative RNA content. The spectral similarities of newborn and adult microsomal brain RNA have been demonstrated by Dingman and Sporn(10).

Results and discussion. The base composition of the ribosomal RNA of newborn and adult rat brain is shown in Table I, and represents the averages of 10 pools of brains as described above. The concentration of ribosomal RNA and total brain ribosomal RNA, expressed as absorption units at 260 mμ in 0.05 N HCl is also shown.

Our results indicate that in the developing rat brain there is no change in the molar percentage of the pyrimidine bases but that inverse alterations in adenine and guanine concentrations occur. The values in the adult rat are similar to that obtained by Frontali (12) in rat brain microsomes and by Yamagami *et al*(13) in guinea pig brain ribosomes. Thus the purine:pyrimidine ratio is constant but the G+C/A+U ratio is less in the adult brain falling to 1.52 as compared to 1.65 in the newborn animal. The differences are statistically significant at less than a 1% level of confidence. These results as well as the direction of change are similar to the values reported by Hydén and Egyhazi(7) in cortical neurons before and after a learning situation. There is also a decrease in ribosomal RNA concentration in the brain from 25.9 to

12.0 O.D. units (260 mμ/g of wet brain tissue) in the adult and neo-natal brain respectively. However, as the mass of the brain increases from 240 mg at birth to 1.55 g in the adult, the total ribosomal RNA is increased about 3-fold from 6.3 to 18.5 O.D. units. A fall in the concentration of brain RNA in the adult rat has been reported(14), and similar results on developing brain in other species have been observed(15,16).

The difficulties in obtaining RNA in the newborn from cellular sites other than microsomes in quantities sufficient to perform base composition analysis restricted the investigation to this structure. It is not possible to determine whether the changes are confined to any specific RNA fraction or are generalized, or whether localized brain areas would show larger differences. The changes observed indicate that ribosomal brain RNA base composition does vary with the development of that organ. These RNA changes may also result from a suppression of some DNA coding sequence which is functional in embryonic and neo-natal brain, but is not required thereafter. That more pronounced changes may be found in localized areas with specialized function is worthy of further exploration. That specific RNA types may be involved is also to be considered.

Though the ribosomal-RNA concentration decreases in the older brains, there is a net synthesis of this nucleic acid in that brain organelle. It is apparent that this may involve the production of a different population of RNA molecules than that elaborated during embryonic development. Whether information processing plays a role in determining the type of brain RNA that is synthesized or formed would appear to be at the center of this problem.

Summary. In the adult brain the ribosomes have a higher AMP-level and show a commensurate fall in GMP when compared to the newborn brain. There is a 3-fold total increase of ribosomal RNA in whole adult brain from birth, although the concentration of ribosomal RNA is higher in the young animal brain. There is no difference between neo-natal and adult rat brain ribosomes in their molar concentration of the pyrimidine bases.

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