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Authors

BONDY, SC
WAELSCH, H

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RNA POLYMERASE IN THE CENTRAL NERVOUS SYSTEM

S. C. Bondy and H. Waelisch

N. Y. Psychiatric Institute and College of Physicians and Surgeons,
Columbia University, New York, N. Y.

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The role of RNA in protein synthesis, the high rate of protein turnover in the brain (1,2) and the possible involvement of nucleic acids (3) and proteins (2) in the higher functions of this organ, make a determination of cerebral RNA (ribonucleic acid) polymerase of interest. Mammalian RNA polymerase has not been purified but its localization in the nucleus of the cell, as well as its dependence on the presence of the four constituent nucleotides, has been demonstrated (4). We wish to present data on the activity of the enzyme in the whole, mature and immature brain and liver, and in various regions of the brain. The experiments were carried out on rabbits.

Methods

Cerebral and hepatic nuclei were prepared by the method of Siekevitz (5). The tissue was homogenized in 0.88M sucrose, the homogenate being layered over 2.2M sucrose, with subsequent centrifugation at 60,000 x g for 90 minutes. Examination of the resulting pellet under the phase contrast microscope showed a high proportion of intact nuclei with little contamination by other morphological components. The nuclei were suspended in isotonic saline, centrifuged at 1,000 x g for ten minutes, and the precipitate was resuspended in saline for the enzyme assay.

The incubation of the nuclei was carried out for 3 minutes at 37° during which time the RNA polymerase activity was found to be linear. The reaction mixture was similar to that of Weiss (4) the trinucleotide dependent incorporation of C¹⁴ labelled ATP into RNA being determined. The

reaction was stopped by the addition of 100 mg unlabelled ATP (6) followed by 1 mg serum albumin and 8 ml of ice cold 5% TCA to each tube. The precipitate thus formed was separated by centrifugation and washed in cold 5% TCA 3 times, after which the precipitate was dissolved in 1 ml 70% formic acid, the solution evaporated to dryness on planchets, and counted in a Nuclear Chicago Low Background Counter.

The results were expressed on a DNA basis in order to relate enzyme activity to the number of nuclei present. The DNA was estimated on an aliquot of the nuclear suspension by Burton's (7) modification of the original method of Dische.

TABLE I

Properties of RNA Polymerase from Nuclei of Whole Rabbit Brain.

	RNA polymerase (μ moles adenine incorporated into RNA per mg. DNA)
1. Complete assay system	605
2. 1 - $MnCl_2$	47
3. 1 - $(NH_4)_2SO_4$	85
4. 1 - CTP	116
5. 1 - GTP	93
6. 1 - UTP	85
7. 1 preincubated 15 min. at 37° with 1 γ DNA, 4 μ moles $MgCl_2$	23

1.15 ml incubation mixture contained 0.2ml nuclear suspension, 10 μ moles $MnCl_2$, 80 μ moles tris - HCl pH 8.1, 10 μ moles mercaptoethanol, 20 μ moles NaF, 10 μ moles phosphoenolpyruvate, 200 μ moles $(NH_4)_2SO_4$, 2 μ moles CTP, GTP and UTP, 100 γ pyruvate kinase, 20 γ calf thymus DNA and 0.45 μ C 8- C^{14} ATP (9.1 mC/mM).

Results and Discussion

The enzyme from rabbit brain closely resembled the liver enzyme in its requirements for all four nucleotides, for a divalent metal ion, and high ionic strength (4,8). The enzyme was inactivated by a 15 minute preincubation with 1 γ of DNAase in the presence of magnesium chloride.

Although the in vitro measurements of an enzyme may not reflect its in vivo activity, the data presented here are of interest for a variety of reasons (for representative results see Table II).

Polymerase activity of the nuclei from whole brain is considerably

TABLE II
RNA Polymerase of Brain and Liver (Adult and Newborn).

Brain	μmoles of adenine incorporated into RNA per mg DNA	
	Adult	Newborn
Cortex (cerebral)	1010	520
Corpus callosum	430	
Thalamus, hypothalamus	514	

Liver	240	150

Incubation conditions as in Table I. Values corrected for blank obtained after incubation in the absence of CTP, UTP, GTP.

higher than that from liver. This may be a reflection of a rate of protein turnover in the neurons at least as high as that in liver cells, a possibility suggested also by results on the incorporation of amino acids into ribosome preparations (9). Nuclei prepared from the cerebral cortex were much more active than those from other areas of the brain. If the assumption is made that glial nuclei, irrespective of their regional origin, have the same enzymatic activity, the polymerase activity of the cortical neurons would be considerably higher than the values obtained in the mixed populations of nuclei. The low polymerase activity of the regions of the brain other than the cortex are of interest since some of these regions also contain a considerable number of neurons. It appears that the correlation between high polymerase activity is not with neurons in general but rather with the cortical neurons. It should be noted that in the immature brain, polymerase activity is lower than that in the mature brain, a property which this enzyme shares with many other enzymes which increase in their concentration and activity during the maturation of the brain (10). Experiments in which C¹⁴ UTP was used in place of C¹⁴ ATP gave essentially the same results.

Summary

RNA polymerase in cerebral cortex is higher than in other regions of the rabbit brain. The average activity of the brain nuclei is higher

than that of the liver nuclei. During development, the activity of RNA polymerase increases with maturation.

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