EFFECT OF SIZE OF POLY(A) SEGMENTS ON INHIBITION OF HETEROGENEOUS NUCLEAR RNA SYNTHESIS IN THE RAT BRAIN BY CORDYCEPIN

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Cordycepin (3'-deoxyadenosine) is a specific inhibitor of post-transcription polyadenylation of RNA [4] and is widely used in experiments on intact [10, 12] and virus-infected [7, 8] eukaryote cells. The literature on cordycepin contains various references to the unequal sensitivity of biosynthesis of certain fractions of cytoplasmic nonribosomal and heterogeneous nuclear RNA (hnRNA) to this inhibitor [1, 6].

The authors investigated the effect of cordycepin on biosynthesis, in rat brain cells, of hnRNA fractions containing and not containing poly(A)—poly(A^+)hnRNA and poly(A^-)hnRNA segments and also of poly (A^+)hnRNA fractions differing in the size of their 3'-terminal poly(A) segments and, consequently, in their affinity for poly(U)-sepharose.

EXPERIMENTAL METHOD

The cordycepin was obtained from PL Biochemicals, USA, the 8-^{14}C-adenine (4.81×10^9 Bq/mmole) from Amersham Corporation, England, poly(U)-sepharose from Pharmacia, Sweden, and DNase free from contamination with RNase and A and T_1 ribonucleases from Worthington, USA.

Different doses of cordycepin were injected intraperitoneally into 3-month-old male WAG33/Sto albino rats, and 1.5 h later [^{14}C]adenine was injected in a dose of 6.66×10^6 Bq/100 g body weight. The animals were decapitated 2 h after injection of the isotope. Nuclei were isolated from brain tissue by the method in [11]; hnRNA was isolated by the method in [3] using DNase treatment of the nuclear material. Next, poly(A^-)hnRNA was separated from total hnRNA, the poly(A^+) fraction was collected, and the number of components differing in the size of their oligo(A) regions and poly(A) segments were isolated from it by consecutive thermal elution from poly(U)-sepharose [5]. The fraction with minimal affinity for poly(U)-sepharose was eluted first, by washing the columns with solution containing 25% formamide at 25°C. The remaining fractions were then eluted with a sudden change in the conditions of elution in the following order: 25% formamide (35°C), 25% formamide (45°C), 25% formamide (55°C), 90% formamide (55°C) [5]. To characterize the resulting fractions of poly(A^+)hnRNA poly(A) segments were isolated from them by treatment with ribonucleases A and T_1 and the size of the molecules of these segments was determined by electrophoresis in 12% polyacrylamide gel in the presence of 7 M urea, pH 7.60 [9].

EXPERIMENTAL RESULTS

The degree of inhibition of biosynthesis of hnRNA fractions was determined by taking incorporation of [^{14}C]adenine into the corresponding brain hnRNA fractions from animals of the control group, which did not receive injections of cordycepin, as the zero level of inhibition. As Fig. 1 shows, cordycepin virtually did not inhibit biosynthesis of poly(A^-) and oligo(A^+)hnRNA of the cells studied. This fact agrees with the existing view that cordycepin has no action on transcription of the eukaryote genome [4, 10]. Resistance of biosynthesis of hnRNA containing oligo(A) sequences containing 20-30 nucleotides to cordycepin can evidently be explained by the transcription origin of these fragments, located in the inner regions of the hnRNA molecule [3]. This fraction of oligo(A^+)hnRNA is known to consist of molecules with no homopolyribonucleotide segments at the 3'-end [3].

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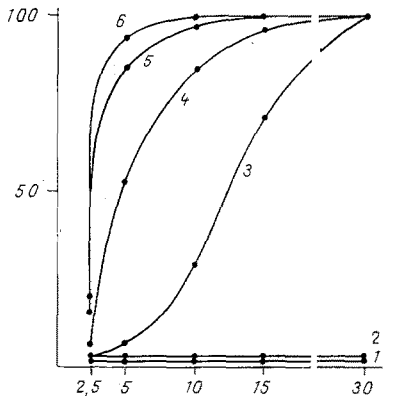

Fig. 1. Effect of cordycepin on biosynthesis of some rat brain hnRNA fractions (n = 10). 1) Poly(A^-)hnRNA; 2) oligo(A^+)hnRNA with oligo(A) measuring 20-30 nucleotides; 3-6) poly(A^+)hnRNA with poly(A) segments measuring 40-60, 60-110, 110-170, and 170-210 nucleotides respectively. Abscissa, dose of cordycepin (in mg/100 g body weight); ordinate, inhibition of hnRNA biosynthesis (%). The optimal configuration of the curves was determined by means of Fortran PAD programs containing special instructions for automatic determination of the values of Student's criterion of significance. Ordinates of points are equal to statistically significant values of means; for all points $P < 0.05$. Computer analysis of the data and construction of curves undertaken by IBM-360 computer with graph-plotter of the "Oristo" (USA) system.

TABLE 1. Characteristics of Rat Brain hnRNA Fractions Whose Biosynthesis is Sensitive to Cordycepin (n = 10)

Fraction (conditions of elution from poly(U)-sepharose	Size of 3'-terminal poly(A) segments, number of nucleotides	Content in total poly(A^+)-hnRNA (M ± m)
25% formamide 35 °C	40—60	9,3±1,0
25% formamide 45 °C	60—110	14,1±1,6
25% formamide 55 °C	110—170	47,6±3,9
90% formamide 55 °C	170—210	29,0±2,1

Legend. Statistical significance of results estimated nonparametrically by the traditional Fisher—Student method. For all values in this Table $P < 0.05$.

It was also found that biosynthesis of rat brain cell hnRNA with short 3'-terminal poly(A) segments was least sensitive to cordycepin. Small doses of the antibiotic (5 and 10 mg/100 g body weight), for instance inhibited biosynthesis of hnRNA containing poly(A) segments 40-60 nucleotides long, by only 5-6 and 26-28% respectively. These same doses of the inhibitor, moreover, were sufficient to produce practically complete inhibition of biosynthesis of hnRNA whose poly(A) segments include 170-210 nucleotides in their composition (Fig. 1).

It can be tentatively suggested that the dimensions of the poly(A) segments are programmed by the "final" size (or something close to it), i.e., the size, after achievement of which further polyadenylation of this hnRNA ceases. In that case, and given a low intracellular concentration of cordycepin, the smaller the "final" size of this chain the less likely the cordycepin is to be incorporated into the growing poly(A) chain.

The mechanism of action of cordycepin is that, since it possesses affinity for poly(A)-polymerase, this inhibitor (3'-deoxyadenosine) is incorporated into the growing poly(A) chain, which terminates the synthesis of that chain because it has no OH group at the 3'-atom of ribose [4, 10]. The formation of the 3'-terminal poly(A) segment of hnRNA however, takes place through consecutive addition of several transformed oligo(A) to the 3'-oligo(A)-"primer" [2, 10]. The use of a small dose of cordycepin had the result that by no means all of the free oligo(A) nucleotides are "attacked" by cordycepin. As a result the cordycepin-containing oligo(A) [3'-deoxy-oligo(A)], the number of which is small, are incorporated mainly into the growing chains of those poly(A) segments whose synthesis requires the greatest number of acts of growth of thechain, i.e., into poly(A) with the largest "final" size.

The low relative content of hnRNA with short poly(A) segments in total poly(A^+)-hnRNA (Table 1) is also evidence in support of our suggested model of the molecular mechanism of selectivity of the effect of small doses of cordycepin: a decrease in the number of hnRNA molecules with small "final" size of their poly(A) segments reduces the likelihood that 3'-deoxy-oligo(A) segments, the content of which in the reserves of unmodified oligo(A) is very small, will take part in the biosynthesis of these segments.

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ENDOGENOUS PHOSPHOLIPASE HYDROLYSIS IN THE CEREBRAL CORTEX DURING DEVELOPMENT OF EPILEPTIC ACTIVITY IN RATS

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The development of epileptic activity (EA) in the CNS is considered to be associated with structural changes in neuron membranes [5]. Structural and functional changes in biomembranes are induced by changes in the physicochemical state of their lipid component [15]. Lipid peroxidation (LPO) is one of the processes that determines the properties of membrane lipids [1]. The writers previously found activation of LPO in membrane fractions of the cerebral cortex during the development of EA [5, 6] and they showed that uncompensated intensification of LPO plays a pathogenetic role [5, 7].

Besides peroxidation, another factor determining the chemical composition and phase state of membrane lipids is the intensity of endogenous phospholipase hydrolysis (PLH) [8]. There

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