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IN VITRO BIOSYNTHESIS, ISOLATION, AND ACTIVITIES OF NUCLEAR GLYCOPROTEINS

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It has been shown by HPLC, electrophoresis, etc. , that, in vitro, the nuclei of neurons of rabbit and cow brains synthesize two glycoproteins (M 25-30 kDa and 10-15 kDa). The influence of some neurotropic compounds on the kinetics of the nuclear protein synthesis has been investigated. New inhibitors of this biosynthesis have been found.

The main functions of the cell nuclei of eukaryotes consist in the preservation of genetic information and the expression of genes with the subsequent transfer of this information to the cytoplasm and also to daughter cells as the result of replication. This scheme excludes the synthesis of proteins by cell nuclei, although at the end of the sixties it was shown that isolated nuclei and chromatin from eukaryote cells synthesize small amounts of proteins [1-6].

In the present communication we give new results on features of the biosynthesis of proteins by nuclei of the nondividing cells of brain neurons in vitro, and some chemical and physicochemical properties of the products of this process.

The biosynthesis of protein was investigated by measuring the level of inclusion of [^{14}C]lysine by the nuclei in a medium containing 0. 1 M glucose, 25 mM $MgCl_2$, 65 mM NaCl, 2 mM $CaCl_2$, and 1 μCi of [^{14}C]lysine. The kinetics of inclusion depended on the temperature nd on the concentration of the reserve components contained in the nuclei.

The inclusion of [^{14}C]lysine in the nuclear proteins took place fairly intensively . In the first 15 min 30% of the total activity was included in the composition of the protein synthesized, during the 15th to 45th min, 50%, and during the 45th to the 60th min, 90%. After this, the level of inclusion remained unchanged. We obtained a similar pattern with nuclei from the neurons of the cow brain (Fig. 1).

The nonuniformity of the inclusion of the labeled amino acid is possibly connected with the simultaneous synthesis of several protein molecules. The neuronal nuclei continue to include [^{14}C]amino acids in vitro even in the absence of the endogenous factors used for the ribosomal biosynthesis of protein (enzymes, a mixture of 18 unlabeled amino acids, ATP, GTP, etc.). On their addition to the incubation medium, the intensity of protein biosynthesis by the nuclei changed only slightly, which confirmed the results of other authors [7]. The procedure for the isolation and purification of the nuclei used in our investigations apparently did not lower their functional activity in the process of protein synthesis.

The facts given permit the conclusion that isolated cell nuclei from rabbit-brain neurons are capable of synthesizing proteins in an in vitro system under conditions different from those of ribosomal synthesis (Table 1).

It must be mentioned that the protein-synthesizing activity of the nuclei (PSAN) of brain neurons depends on the time from their isolation: after a day it had fallen by 9%, after two days by 20%, and after seven days by 58%. To find the distribution of labeled amino acids in the nuclei after the end of protein biosynthesis, we made an autoradiographic study using [^{3}H]leucine. It was found that the [^{3}H]leucine radioactivity within the nuclei was distributed over more than 10 sections.

On the basis of these facts it may be assumed that protein synthesis in brain neuron cell nuclei takes place at several points and that autonomic processes of activation of the amino acids of the neuronal proteins function in the nuclei.

To investigate the chemical nature of the proteins synthesized in the nuclei, the extracted, desalted, and freeze-dried material was fractionated on a previously calibrated column of Sephadex G-50 fine. Two radioactive peaks, corresponding to two fractions of nuclear protein, were detected (Fig. 2). The molecular masses of the labeled nuclear proteins were determined by electrophoresis as 25-30 kDa (I) and 10-15 kDa (II). The fractions corresponding to these peaks were combined,

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TABLE 1. Conditions for the in vitro Synthesis of Proteins*

On ribosomes	In a nucleus
0.5 ml of a suspension of ribosomes in 45 mM Tris buffer containing 7.5 M $MgCl_2$, 0.12, M KCl, and 0.075 M NaCl, pH 7.4.	0. 5 ml of a suspension of nuclei in 0.1 M sodium phosphate buffer, pH 7.4
	0. 1 M glucose
1. 0 mM ATP	0. 025 M $MgCl_2$
0. 6 mM GTP	0. 065 M NaCl
5 mM phosphoenol pyruvate	2 mM $CaCl_2$
0. 04 M of a nixture of unlabeled amino acids	1 μCi of a [^{14}C]amino acid
Cell juice	
5 μCi of a [^{14}C]amino acid	

*The concentrations of salts, amino acids, and enzymes necessary for the optimum synthesis of proteins in the nucei and the ribosomes are given.

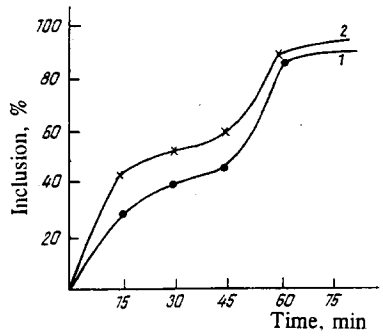

Fig. 1. Kinetics of the inclusion of [^{14}C]lysine by the nuclei of cow (1) and rabbit (2) brain neurons.

freeze-dried, and separated by HPLC (Fig. 3). Several optical-density peaks were obtained, two of which (test-tubes 4 and 14) were radioactive. The radioactive peaks were separated preparatively and their homogeneity was determined with the aid of electrophoresis. The results of an electrophoretic study of the total proteins isolated (Fig. 4) showed a large number of protein bands After electrophoresis, the gel was cut into strips 0.2 mm wide, which were dissolved in a mixture of hydrogen peroxide and concentrated ammonia (9.9:0.1, v/v) and placed in scintillation liquid. Radioactivity was detected in two protein components, with M 25-30 and 10-25 kDa, identical with the protein components isolated by HPLC (bands 3 and 4). These results confirmed our opinion that the synthesis of more than one protein takes place in the nucleus. Band 5 shows the autoradiographic zones of the new nuclear proteins. The staining of the protein zones by Coomassie and periodic acid permitted the assumption of their glycoprotein nature.

The influence of neutropic drugs on the efficiency of synaptic transfer has already been fairly widely studied. However, the action of neurotropic drugs and peptides on the state of nuclear membranes and protein synthesis conjugated with them is of particular interest. We have already reported the action of some neurotropic agents on the kinetics of the biosynthesis of nuclear proteins in an in vivo system [8]. Thus, Fig. 5, *a*, shows that the action of neurotropic agents is not restricted to the cell level but extends as far as the cell nucleus, as a result of which the rate of nuclear biosynthesis of proteins changes. As can be seen from Fig. 6, such substances that we used as cocaine, strychnine, chlorpromazine, and cobra venom were inhibitors of this process.

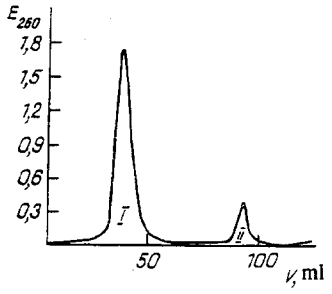

Fig. 2

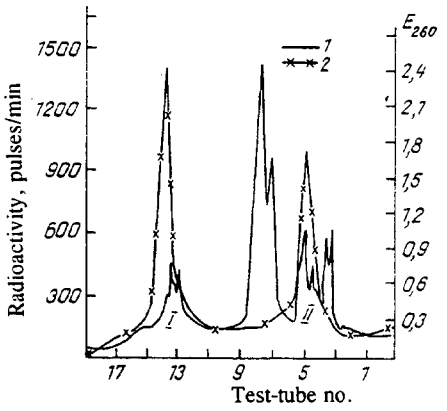

Fig. 3

Fig. 2. Fractionation on Sephadex G-50 of the proteins synthesized by brain neuron nuclei (0.05 ammonium acetate buffer, pH 6.06).

Fig. 3. Separation of fractions I and II on columns of Nucleosil (4.6 × 250 mm, 5 μm) and Zorbax PSM 60 (0.62 × 60 cm) using an acetonitrile gradient. 1) E_{260}; 2) radioactivity, pulses/min.

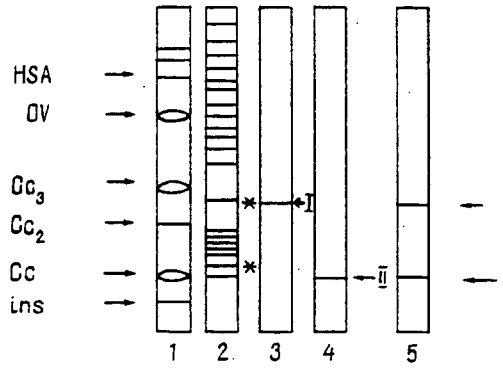

Fig. 4. Electrophoretogram of the fractions synthesized by rabbit brain neuron nuclei: 15% PAAG in the presence of Na SDS, stained with Coomassie. 1) Marker proteins; the arrows show the positions of the following marker proteins: HSA) human serum albumin (68 kDa); OV) ovalbumin (45 kDa); Cc) — cytochrome C (12.4 kDa); ins) pancreatic gland insulin (5.7 kDa); Cc_2) dimer of cytochrome C; Cc_3) trimer of Cc; 2) total nuclear proteins; 3) protein fraction I (the arrows show the labeled proteins); 4) protein fraction II; 5) autoradiographic revelation (labeled sections shown by arrows); *) glycoprotein zone.

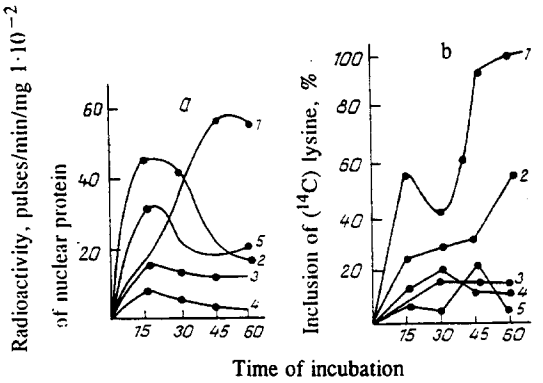

Fig. 5. Kinetics of the biosynthesis of nuclear proteins in neuronal cells. *a*) Rabbits (control and experimental animals) 15 min after the administration of the substances: 1) control, 0. 9% NaCl; 2) cobra venom, 0.1 mg/kg; 3) chlorpromazine, 1.4 mg/kg; 4) strychnine, 0.4 mg/kg; 5) cocaine, 8 mg/kg; 6) cow brain in an in vitro system; 1) control; 2) heroin, 10 μg; 3) heroin, 15 min — 20 μg; 30 min — 40 μg; 45 min — 60 μg; 60 min — 80 μg; 4) enkephalin, 5 μg; 5) enkephalin, 15 min — 20 μg; 30 min — 40 μg; 45 min — 60 μg; 60 min — 80 μg.

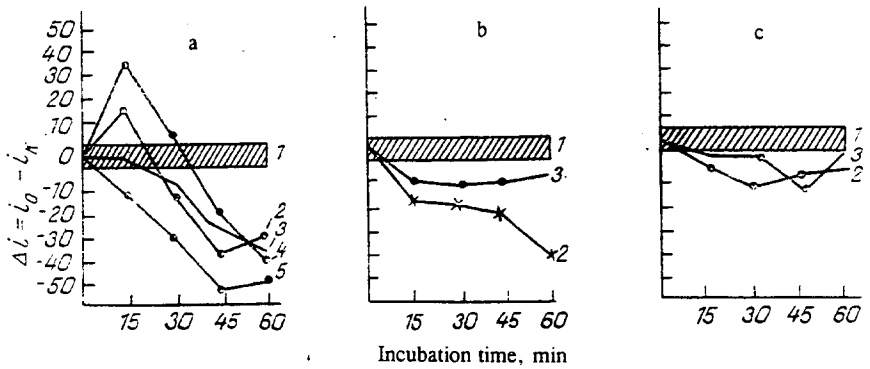

Fig. 6. Kinetic measurements of differential radioactivity magnitudes (relative to a control in action on the PSAN of neuronal cells: *a*) of rabbits 15 min after the administration of the substance: 1) control; 2) cobra venom, 0. 1 mg/kg; 3) chlorpromazine, 1.4 mg/kg; 4) strychnine, 0.4 mg/kg; 5) cocaine, 8 mg/kg; *b*) of the cow brain under the action of heroin: 1) control; 2) 10 μg; 3) 20 μg after 15 min; 40 μg after 30 min; 60 μg after 45 min; 80 μg after 60 min; *c*) of cow brain under the action of enkephalin: 1) control; 2) 5 μg; 3) 20 μg after 15 min; 40 μg after 30 min; 60 μg after 45 min; 80 μg after 60 min.

We studied the mechanism of the action of heroin and enkephalin in an in vitro system (Fig. 5, *b*). Heroin in a concentration of 10 μg likewise suppressed the synthesis of nuclear protein on incubation for 60 min by almost 50%. At a heroin concentration of 20 μg and incubation for 30 min, of 40 μg for 30 min, 60 μg for 45 in, and 80 μg for 60 min the suppressive action reached more than 80%, i.e., there was a pronounced suppression of nuclear synthesis. We observed a similar pattern in the case of enkephalin. The action of the substances tested on the kinetics of the protein-synthesizing activity (PSA) was traced more clearly in an analysis of differential graphs of the changes in the dynamics of the inclusion of [^{14}C]lysine into the nuclear protein in comparison with a control (Fig. 6).

The study of the kinetic laws of the change in the functional activity of nuclei of neuronal cells under the influence of neurotropic drugs may clarify some aspects of the process of information transmission from the nuclear membrane and the coding in the long-term memory of the neuronal cell [9].

The PSAN reflects a reactive state of the nuclear membrane. The interaction of the agents tested with the receptors of the synaptic membranes may either increase the concentration of nonpeptide mediators (cyclic nucleotides) or decrease it, which affects the reactivity of the nuclear membranes and, as a result, changes the level of synthesis of proteins.

To determine the biological activities of nuclear glycoproteins, we attempted to ascertain their role in three ways; namely by studying: 1) the influence of nuclear glycoproteins on the inclusion of labeled amino acids by brain neuron nuclei (i.e., on protein synthesis); 2) the action of the glycoproteins on the transcriptional activity of brain neuron nuclei; and 3) the effect of the glycoproteins on the motive activity (MA) and behavioral reactions of animals.

On analyzing the results obtained, it may be concluded that the action of the glycoproteins, on the one hand, suppresses protein synthesis in isolated nuclei and the RNA polymerase activity of the nuclear chromatin in in vitro systems, and, on the other hand, changes the MA and the behavioral reactions of animals. This indicates that biologically active glycoproteins are synthesized in brain neuron cell nuclei. Nevertheless, so far, we are unable reliably to evaluate the basic function of these nuclear proteins, the determination of this being the aim of our further investigations.

EXPERIMENTAL

The work was conducted on brain neurons of rabbits from one litter and a cow brain. The cow brain was obtained immediately after slaughter in the Tashkent meat combine. The nuclei were isolated from the brain neuron cells by a known method, and the synthesis of protein in the isolated nuclei was carried out as described in [8]. The labeled nuclear proteins were precipitated from the incubation medium with 5% TCAA solution. The precipiate was separated off by centrifugation (15 min at 3000 rpm) and was extracted with a mixture of 0.025 N Tris buffer and 0.192 M glycine buffer, pH 8.3. For desalting, the proteins were dialyzed against water and freeze-dried.

Gel Filtration of the Proteins. The molecular masses of the labeled proteins from the brain neuron nuclei were determined by gel filtration in 0.05 M ammonium acetate buffer, pH 6.06, on a column (1.1 × 102 cm) of Sephadex G-50 (fine, Pharmacia, Sweden) that had been calibrated beforehand with BSA, soybean trypsin inhibitor, and cytochrome C (Reanal, Hungary). Fractions with a volume of 4 ml were collected, and 0.1-0.2-ml samples were counted in a Beckman LS-230 liquid scintillation counter (USA) containing 10 ml of scintillation liquid and 3 ml of absolute alcohol.

Samples were collected, and, after freeze-drying, were reseparated. The labeled nuclear proteins were obtained on a Du Pont 8800 HPLC instrument (USA), using columns of Nucleosil (4.6 × 150 mm, 5 μm) and Zorbax PSM 60 (0.52 × 60 cm).

Protein radioactivities were counted in a Beckman LS-230 liquid scintillation counter (USA) in a mixture containing 10 ml of ZhS-8. The electrophoretic analysis of the nuclear product was conducted in 15% PAAG containing 0.1% of sodium dodecyl sulfate [10].

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