It has been shown [6, 11] that Mo^{++} is a component of the active center of XOase. There is evidence that the enzyme is activated by molybdenum salts [2-4]. In the present experiments administration of ammonium molybdate to the animals was followed by significant activation of XOase, accompanied by a parallel and significant increase in PDE activity (Table 2).

The data described above thus show that there is close interaction between enzymes of the XOase and PDE systems. It can be tentatively suggested that PDE is regulated by the dynamic concentration of endogenous xanthines which, in turn, is determined by the intensity of reactions of the terminal stages of purine metabolism. Pharmacological action on PDE through the XOase–xanthines system may be an important way of modulating the concentrations of cyclic AMP and cyclic GMP in the tissues in clinical and experimental medicine.

LITERATURE CITED

1. D. R. Beritashvili and K. A. Kafiani, Vopr. Med. Khimii, No. 3, 322 (1975).
2. A. I. Kazachenko, L. S. Vartanyan, and É. M. Gonikberg, Biokhimiya, 36, 27 (1971).
3. V. V. Koval'skii and I. E. Vorotnitskaya, Dokl. Akad. Nauk SSSR, 187, 1422 (1969).
4. S. E. Severin, P. P. Filippov, and G. A. Kochetov, Usp. Sovrem. Biol., 69, 423 (1970).
5. L. A. Tiunov, N. M. Petushkov, and T. R. Koroleva, Byull. Éksp. Biol. Med., No. 5, 34 (1974).
6. E. Di Renzo, E. Kaleita, J. Oleson, et al., J. Am. Chem. Soc., 75, 753 (1953).
7. L. Dietrich, J. Biol. Chem., 211, 79 (1954).
8. P. Feigelson, M. Feigelson, and T. Wood, Science, 120, 502 (1954).
9. G. Litwack, J. Bottwell, J. Williams, et al., J. Biol. Chem., 200, 303 (1953).
10. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
11. B. Mackler, H. Mahler, and D. Green, J. Biol. Chem., 210, 149 (1954).
12. G. Poch, Arch. Exp. Pathol. Pharmak., 268, 272 (1971).

TRANSLATION OF MOUSE INTERFERON mRNA IN A CELL-FREE SYSTEM

U. Kiessling, T. M. Sokolova, and F. I. Ershov

UDC 612.429.015.3:547.963.32:576.858.095.383

KEY WORDS: interferon; synthesis; mRNA.

Interferon is an inducible protein with high antiviral activity (> 10^9 inhibitory units/mg protein). Progress in the study of the mechanisms of synthesis and action of interferon is largely bound up with the development of methods of testing its messenger RNA (mRNA). At the present time both whole cells and cell-free systems of protein synthesis are used for translation of interferon mRNA [2]. The latter open up new opportunities for the study of fine mechanisms of regulation of synthesis and action of interferon at the translation level.

Of the known eukaryotic translation systems the most effective known is a cell-free system consisting of rabbit reticulocyte lysate, in which low concentrations of mRNA (1-5 μg/ml) can be translated in the presence of ribosomal RNA, so that total preparations can be used. Treatment with micrococcal nuclease destroys endogenous templates and enables a system dependent upon added exogenous mRNA to be obtained [5].

This paper gives the results of a parallel study of translation of mouse interferon mRNA in a cell-free system from rabbit reticulocyte lysate and in tissue culture cells.

D. I. Ivanovskii Institute of Virology, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR V. M. Zhdanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 90, No. 12, pp. 675-677, December, 1980. Original article submitted June 6, 1979.

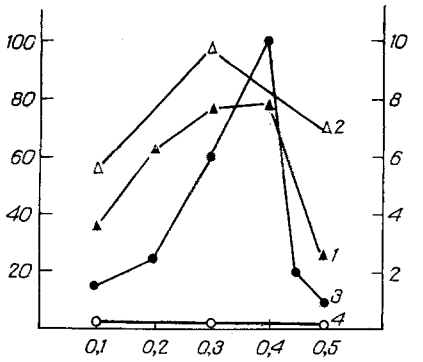

Fig. 1. Incorporation of leucine-^{3}H into acid-insoluble material after addition of RNA from L-929 cells, treated (1) and untreated (2) with poly(I)·poly(C) to a cell-free system. Abscissa: RNA concentration (in mg/ml); ordinate: on left – interferon titer (in units/0.1 ml) in RNA translation product from L-929 cells treated (3) and not treated (4) with poly(I)·poly(C); on right – radioactivity in cpm·10^2.

TABLE 1. Species-Specificity of Interferon Synthesized in a Cell-Free System

Cells	Antiserum against mouse interferon	Interferon titer, units/0.1 ml
L-929	–	800
L-929	+	<100
CEF	–	<100

Legend. Interferon activity determined by titration on L-929 and CEF cells relative to CPA with VEE virus in the absence and presence of antiserum against mouse inteferon.

EXPERIMENTAL METHOD

RNA containing interferon mRNA was isolated from cells of continuous line L-929 5 h after induction with poly(I)·poly(C) in the presence of cycloheximide, as described by the writers previously [1]. Parallel preparations of control RNA were obtained from untreated cells. The cells were disintegrated in hypotonic buffer (20 mM Tris-HCl, pH 7.6, 10 mM NaCl, 1 mM EDTA, 1.5 mM Mg acetate) with 0.1% Triton X-100. The nuclei were removed by centrifugation. RNA was isolated with a mixture of phenol and chloroform (1:1) saturated with STE buffer (130 mM NaCl, 100 mM Tris-HCl, pH 9.0, 1 mM EDTA) in the presence of 1% sodium dodecylsulfate. To remove possible double-helical RNA present as impurities, precipitation with 2 M ammonium acetate was used. The RNA residue was washed twice or three times with 70% ethanol. The cell-free system was obtained from rabbit reticulocyte lysate as described previously [3] with some modifications. Anemia was induced in rabbits by bleeding four times (10-15 ml/kg body weight). Blood was collected 24 h after the last bleeding from an auricular vein and a reticulocyte lysate was prepared. The lytic solution contained 50 μM hemin and 2 mM dithiothreitol. The reaction mixture contained: 100-120 mM potassium acetate, 0.5 mM magnesium acetate, 1 mM dithiothreitol, 2 mM ATP; 0.2 mM GTP, 15 mM creatine phosphate, 100 μM each of 19 amino acids, 200 μM spermidine, and 60% reticulocyte lysate. Protein synthesis was determined from the incorporation of leucine-^{3}H (60 Ci/mmole) or leucine-^{14}C (380 mCi/mmole) in the acid-insoluble material. Creatine phosphokinase (50 μg/ml) was added immediately after lysis of the reticulocytes. Treatment with micrococcal nuclease was carried out in the presence of Ca^{++}. The reaction was stopped by addition of EGTA to a concentration of 2 mM. RNA preparations from mouse cells were added to the system in concentrations of between 0.1 and 0.5 mg/ml.

TABLE 2. Translation of Interferon mRNA in Cell Cultures

Conditions of treatment of cells	Interferon titer, units/ml	
	L-929	CEF
Medium No. 199 with DEAE-dextran	8	4
Medium No. 199 with amphotericin B	2	8
0,5 M NaCl	8	2
PBS with $CaCl_2$	4	8

Chick fibroblasts and L-929 transplantable mouse cells were used for translation of interferon mRNA in cell culture. RNA was diluted to a concentration of 30-50 μg/ml in the following solutions: in medium No. 199 containing 100-200 μg/ml of DEAE-dextran or 2 μg/ml amphotericin B; 0.5 M NaCl and PBS buffer containing 30 mM $CaCl_2$.

Before addition of RNA the cells were treated for 2 h at 37°C with actinomycin D (1-2 μg/ml). RNA was added to the cell monolayer in penicillin flasks in a dose of 0.2 ml; adsorption continued for 20-60 min. After incubation for 5 h at 37°C interferon was determined in the culture medium.

Interferon synthesized in the cell-free system was titrated by a micromethod on plastic slabs [7], whereas interferon synthesized in cell cultures was titrated in penicillin flasks by determination of its cytopathic action (CPA) on L-929 cells with Venezuelan equine encephalitis (VEE) virus. The interferon was incubated with antiserum against mouse interferon (100 units) at 20°C for 30 min. Highly purified anti-interferon serum was generously provided by Dr. G. J. Galasso (USA).

EXPERIMENTAL RESULTS

Messenger RNA from mouse cells treated with poly(I)·poly(C) interferon inducer stimulated incorporation of labeled amino acids into the acid-soluble fraction in lysates of rabbit reticulocytes treated with micrococcal nuclease, which increased over the concentration range of RNA from 0.1 to 0.4 mg/ml and then fell sharply (Fig. 1). The translation product of mRNA from the induced cells contained mouse interferon, the quantity of which also increased with an increase in RNA concentration, although not directly proportionally. In an RNA concentration of 0.4 mg/ml the greatest incorporation of labeled amino acids and maximal synthesis of interferon were observed, the latter amount to 100 units/0.1 ml of 50 units/ml/μg RNA added to the system. In higher RNA concentrations both the incorporation of radioactive precursors and the interferon content fell sharply. This result can evidently be explained by the inhibitory action of ribosomal RNA on the translation system when total preparations of RNA were used in high concentrations, as has been shown in the case of globin synthesis in reticulocyte lysate [6]. After addition of control RNAs (from noninduced cells) to the system no mouse interferon was found in the translation product.

To determine the species-specificity of interferon synthesized in the cell-free system its activity was determined on homologous L-929 mouse cells in the presence of antiserum against mouse interferon and on heterologous cells (chick embryonic fibroblasts – CEF). It follows from Table 1 that the synthesized interferon possessed antiviral activity only on mouse cells (800 units/ml). Activity of the interferon on mouse cells was neutralized by 100 units of anti-interferon serum. The product obtained in reticulocyte lysates in response to injection of RNA from cells induced with poly(I)·poly(C) thus had the properties of mouse interferon.

In parallel experiments the same RNA preparations (30-50 μg/ml) were translated in L-929 and CEF cultures in the presence of substances increasing the permeability of the cells and adsorption of RNA on them. It will be clear from Table 2 that titers of interferon translated in the cells were low: 2-8 units/ml. The efficiency of translation in the cell cultures per microgram RNA was on average only one-hundredth of that in the cell-free system (2-8 units/ml/μg RNA). This was due to poor penetration of RNA into the cells and to the action of nucleases on them. The efficiency of translation of interferon mRNA in reticulocyte lysates was 40% of the most active system: oocytes of the frog Xenopus laevis [4]. Now that biologically active interferon can be obtained in a cell-free system from rabbit reticulocyte lysate, it is possible to study the as-yet unexplained mechanism which regulates the production and action of interferon.

LITERATURE CITED

1. F. I. Ershov et al., Acta Virol., 23, 32 (1979).
2. T. M. Sokolova and U. Kiessling, Antibiotiki, No. 12, 1112 (1978).
3. T. Hunt et al., J. Mol. Biol., 66, 471 (1972).
4. B. Lebleu et al., Biochem. Biophys. Res. Commun., 82, 665 (1978).
5. H. R. B. Pelham et al., Eur. J. Biochem., 67, 247 (1976).
6. H. Suzuki, J. Biochem. (Tokyo), 83, 1061 (1978).
7. J. Vilcek et al., J. Virol., 10, 614 (1972).

EFFECT OF ACETYLCHOLINE ON Na,K-ATPase OF BRAIN MICROSOMES FROM RATS OF DIFFERENT AGES

R. I. Potapenko

UDC 612.82.015.1:577.152.361-053

KEY WORDS: aging; brain; ATPase; acetylcholine.

The process of aging is characterized by limitation of the functional capacity of various organs and tissues, including the brain [8]. There is reason to suppose that an important role in the origin of these disturbances is played by the energy deficiency which develops with age.

To study the metabolic mechanisms of age changes in the brain, an important contribution could be made by an examination of the state of transport Na,K-ATPase in old age and changes in its activity under the influence of acetylcholine (ACh). It has been shown that ACh inhibits Na,K-ATPase in brain microsomes [3] and synaptosomes [2, 4], and thus helps to regulate excitation processes in the neuron. There have been isolated studies of brain ATPase activity in the late stages of ontogeny [5-7], but these were conducted on the whole brain without differentiation of its parts. The results of these investigations have proved contradictory.

The object of this investigation was to determine Na,K-ATPase activity in microsomes of the cerebral cortex and the effect of ACh on it in animals of different ages.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats aged 6-7 months (mature) and 25-26 months (old). The animals were decapitated, the brain was removed as quickly as possible, and the cortex was separated from the white matter of the hemispheres. Cortical tissue was homogenized in 0.32 M sucrose with 0.01M Tris-HCl buffer, pH 7.4, for 30-40 sec in a glass homogenizer with Teflon pestle. Microsomes were obtained by differential centrifugation (40,000g, 1 h) of the supernatant obtained after removal of nuclei and mitochondria. The fraction thus isolated was resuspended in isolation medium and used after a single freezing and thawing.

Total ATPase activity was determined in medium (final volume 2 ml) containing (in mM): NaCl 100, KCl 20, ATP-Na_2 2, $MgCl_2$ 2, and 150-170 μg microsomal protein or 250-300 μg protein of homogenate. Mg-ATPase activity was determined in an identical solution in the presence of strophanthin K (0.1 mM). Experimental samples contained ACh in a concentration of 6 mM. The reaction (15 min, 37°C) was started by addition of the substrate. It was stopped by the addition of an equal volume of cold 10% TCA. Inorganic phosphorus was determined in the supernatant [11]. Protein was determined by Lowry's method [12], after preliminary destruction of the membrane with 2% sodium deoxycholate.

Na,K-ATPase activity was calculated as the difference between total and Mg-ATPase activity.

Laboratory of Biochemistry and Physiology, Institute of Gerontology, Academy of Medical Sciences of the USSR, Kiev.

(Presented by Academician of the Academy of Medical Sciences of the USSR D. F. Chebotarev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 90, No. 12, pp. 677-678, December, 1980. Original article submitted February 29, 1980.