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ACTION OF PLANT SUBSTANCES ON THE BIOSYNTHESIS OF GLYCOPROTEINS IN NEURONAL NUCLEI

O. Kh. Saitmuratova and V. B. Leont'ev

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The influence of compounds isolated from plants on the level of biosynthesis of proteins by neuronal nuclei of animal brains has been studied in vivo *and* in vitro. *Inhibitors and activators of biosynthesis have been found among these substances.*

One of the methods of revealing differences between the nuclear and the ribosomal synthesis of proteins is the study of the action of exogenous substance on the level of nuclear synthesis.

Investigations of the influence of various antibiotics and alkaloids on the protein-synthesizing capacity of ribosomes are found in the literature in connection with suggested mechanisms of their action [1-3], but the effect of various exogenous substances of this type on the protein-synthesizing activity of the nuclei (PSAN) of neuronal cells in comparison with the ribosomal system has been studied inadequately.

We have established that the cell nuclei of neurons in *in vivo* and *in vitro* systems synthesize glycoproteins under conditions differing from those characteristic for the functioning of the ribosomes of eukaryote cells. The mechanism of this pathway of protein synthesis has not yet been definitively established. We are the first to have isolated the products of nuclear synthesis — two groups of glycoproteins — from the neurons of animal brains and to have studied their physicochemical properties [4-8].

In order to find means of regulating these processes, we have studied the influence of a number of known compounds isolated from plants but not hitherto investigated in this direction, and derivatives of them, including lupinine, lycorine, heroin, ephedrine, cocaine, strychnine, etc., on the level of biosynthesis of proteins by the nuclei of brain neurons.

Among the alkaloids, the quinolizidine type proved to be the most interesting. Their chemical structures have been studied, their conformational states have been determined, and semisynthetic derivatives and analogs of them have been obtained, which considerably facilitated the performance of investigations of their action mechanisms.

Lupinine stimulated the PSAN to the greatest degree, some dose dependence of it being observed (see Table 1). Judging from the results obtained, in a dose of 75 μg/ml alkaloids containing the conformationally labile system of piperidine and lupinine are more active than, for example, anabasine hydrochloride, anabasinyl O-isopropyl phosphate, and anabasinyl O,O′-diisopropyl phosphate.

Under the action of 100- and 200-μg/ml doses of lycorine, which is an inhibitor of ribosomal synthesis, the biosynthesis of protein in the nuclei was stimulated by 15 and 40%, respectively.

Alkaloids for which stable conformations are characteristic suppressed the synthesis of these proteins in the nuclei: ephedrine in a dose of 50 μg/ml, by 59%; heroin in a doses of 10 and 80 μg/ml, by 41 and 88%, respectively; cocaine in a dose of 8 mg/ml, by 94%; and strychnine in a dose of 0.2 mg/kg, by 72%.

As we see, of the substances mentioned above, some stimulate and some suppress the nuclear synthesis of protein.

In the first place, the exogenous substances affect the cellular and nuclear receptors, and they also act on the multienzyme system of the nuclei where protein synthesis takes place.

The rise in the level of protein synthesis in the nucleus in the presence of lupinine and lycorine is probably caused by an increase in the activity of certain enzymes participating in this process. The fall in the level of formation of proteins in the

A. S. Sadykov Institute of Bioorganic Chemistry, Academy of Sciences, Republic of Uzbekistan, Tashkent, fax (3712) 62 70 71. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 781-783, November-December, 1995. Original article submitted November 7, 1994.

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TABLE 1. Action of Some Exogenous Plant Substances on the Intensity of Nuclear Protein Synthesis

Substance	Dose, μg/ml	Inclusion of [^{14}C]lysine, % on control
Lupinine	–	
	5	147*
	25	158*
	50	150*
	75	159*
Anabasine hydrochloride	–	
	5	118*
	25	122*
	50	113*
	75	101*
N- Anabasinyl O-isopropyl phosphite‡	–	
	5	111*
	25	115*
	50	123*
	75	128*
O,O'-Anabasinyl di-O-isopropyl phosphate	–	
	5	131*
	25	143*
	50	146*
	75	138*
Lycorine	–	
	100	115†
	200	140†
Ephedrine	–	
	50	50†
Heroin	–	
	10	59†
	80	12†
Cocaine	–	
	8 mg/kg	6†
Strychnine	0.2 mg/kg	28†

*Activator.

†Inhibitor. As 100% was taken the number of pulses included per minute in the absence of a test substance, averaged over three experiments. The statistical treatment of the results showed significance ($P \leq 0.05$).

‡"phosphate" in text.

nucleus under the action of cocaine, strychnine, ephedrine, and heroin can be explained by their capacity for inhibiting the activity of the multienzymes participating in nuclear protein synthesis.

Thus, the results that we have obtained show different sensitivities of PSAN to the substances studied. A continuation of these investigations may provide a basis for revealing the mechanism of the action of these natural substances and permit their classification according to the nature of their effects on the synthesis of proteins in nuclei.

EXPERIMENTAL

The brains of 30- to 35-day-old rabbits were taken immediately after decapitation. The cell nuclei were isolated by a published method [9], the neuronal cells having first been separated from the glial cells by microdissection and ground manually in a homogenizer with solution A: 0.32 M sucrose, 0.003 M $MgCl_2$, 0.001 M K_2HPO_4, pH 7.4. The nuclei were isolated from the cell homogenate by centrifugation in a sucrose density gradient in a Beckman ultracentrifuge (USA) with a SW-27 rotor at 78,000*g* for 60 min. The supernatant liquid was decanted off, and the deposit containing the neuronal nuclei was washed

with solution A and suspended in 0.25 M sodium phosphate buffer (pH 7.4). The purity and integrity of the nuclei isolated were checked under the microscope.

Protein synthesis was determined from the inclusion of [^{14}C]lysine [10]. All the preparations used were obtained in the A. S. Sadykov Institute of Bioorganic Chemistry, Academy of Sciences of the Republic of Uzbekistan. The substances studied were added to the incubation medium during synthesis in various doses, none of these substances being added to the control variant. The radioactivity of each product obtained was determined in 10 ml of ZhS-8 scintillation liquid on a Beckman LS-230 counter (USA), and the amount of protein was determined in suspensions of whole cells by Lowry's method [11] before incubation.

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