LITERATURE CITED

1. A. S. Pivovarov, E. I. Drozdova, and B. I. Kotlyar, Abstracts of Proceedings of an All-Union Symposium on Biochemistry of Receptor Systems [in Russian], Tallinn (1990), p. 51.
2. F. Haviv, J. D. Ratajczuk, R. W. De Net, et al., J. Med. Chem., **30**, 254 (1987).
3. D. Kim, D. L. Lewis, L. Craziadei, et al., Nature, **337**, 557 (1989).
4. C. R. Pace-Asciak and S. Asorta, Free Radical Biol. Med., **7**, 409 (1989).
5. V. Y. Vanderhoek, R. W. Bryant, and J. M. Bailey, J. Biol. Chem., **255**, 5064 (1980).
6. V. Y. Vanderhoek, R. W. Bryant, and J. M. Bailey, J. Biol. Chem., **255**, 5996 (1980).

DETECTION OF THE SEROTONIN-MODULATED PROTEIN FRACTION AND ITS ROLE IN ORGANIZATION OF PASSIVE AVOIDANCE BEHAVIOR IN RATS

G. G. Gasanov* and A. A. Mekhtiev

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Mediators are known to initiate many intracellular metabolic events in nerve cells, including regulation of the genetic apparatus [2, 9, 14]. For some mediators and, in particular, for serotonin, functional activity has been shown to depend on their control over protein synthesis [11-13]. Most investigations, however, have dealt with the study of the role of mediators in the regulation of total protein synthesis, although there are grounds for considering that they may have a selective action on protein metabolism [5].

The aim of this investigation was to study the effect of serotonin and noradrenalin on metabolism of individual water-soluble protein fractions in the cerebral cortex and to investigate the role of these fractions in passive avoidance behavior in rats.

EXPERIMENTAL METHOD

The effect of mediators on protein metabolism was studied in the following way. Experiments were carried out on male rats weighing 200-280 g. Under pentobarbital anesthesia an area of the skull with the dura mater was removed from the experimental animals above the occipital cortex of the brain bilaterally, and for 40 min a 10^{-3} M solution of serotonin in physiological saline (n = 12), and in another series 10^{-3} M noradrenalin solution (n = 10) was applied for 40 min. Physiological saline was applied to the control animals. After decapitation of the rats, water-soluble proteins were extracted from the occipital regions of the cerebral cortex in 0.01 M phosphate buffer solution (pH 7.2) containing 0.2 M NaCl, and these were fractionated by disk-electrophoresis under nondenaturing conditions in polyacrylamide gel as described by Davis

*Academician of the Academy of Sciences of the Azerbaijan SSR.

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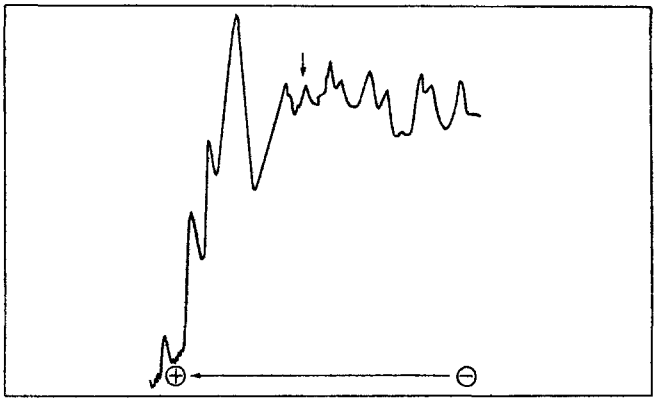

Fig. 1. Densitogram of water-soluble protein fractions separated by disk electrophoresis (arrow indicates fraction No. 6).

TABLE 1. Effect of 10^{-3} M Solutions of Serotonin and Noradrenalin on Content of Fraction No. 6

Series of experiments	Group			
	experimental		control	
	arithmetic mean	scatter	arithmetic mean	scatter
Application of serotonin (n = 12)	0,074*	0,051—0,101	0,085	0,063—0,116
Application of noradrenalin (n = 10)	0,070	0,052—0,080	0,066	0,052—0,076

Legend. *$p < 0.05$

[10]: the concentration of the separating gel was 7% and of the concentrating gel 3%. Electrophoresis was carried out in a Tris-glycine buffer system (pH 8.9). Protein zones separated in the gel were fixed in 12.5% TCA solution, stained with a solution of Coomassie bright blue R-250, and subjected to densitometry in the DM-1 densitometer. The relative content of each fraction was calculated and the significance of differences estimated by Wilcoxon's T test for tied pairs [6]. Immunoglobulins to fraction No. 6 were obtained by immunization of rabbits for 3-4 months (three subcutaneous injections of antigens in Freund's complete adjuvant, at 2-weekly intervals, subsequent injections once a month). Fraction No. 6, obtained by disk-electrophoresis, mixed with gel fragments, was used as the antigens. Samples of 60 ml blood were taken 10 days after the 23rd and last injections from the rabbits, and immunoglobulins were isolated [8]. The presence of antibodies in the solution of immunoglobulins was determined by solid-phase enzyme immunoassay (ELISA), using a 5-point scale [3]. All the tests were duplicated. The immunoglobulins were transferred into physiological saline by gel-filtration and kept at −18°C. Behavioral experiments were carried out on male rats weighing 200-280 g in a passive avoidance chamber, consisting of a light and and a dark compartment, connected by a narrow corridor. Photoelectric transducers, connected to an automatic writer, were mounted in the walls of the corridor. Three groups of animals were formed: 1) intact (n = 17), 2) control (injection of nonimmune gamma-globulins, n = 13), 3) experimental (immunoglobulins to fraction No. 6, n = 16). Under superficial ether anesthesia, the rats were given an injection of 10 μl of the preparations in a concentration of 5 mg/ml into the left lateral cerebral ventricle. The animals were trained 24 h later in passive avoidance, and 48 h after training preservation of the skill was tested (10 min). During training the rats received an electric shock from a current of 0.8 mA applied through the electrified floor in the dark compartment of the chamber. Average probabilities for the groups of discovering rats in different parts of the chamber, the number of times they passed the photoelectric transducers, and the latent periods of visiting and the relative percentage of animals found in the dark compartment of the chamber were calculated from the results of testing. The significance of differences was estimated by Student's t test, the Wilcoxon—Mann-Whitney U-test, and Pearson's chi-square test [6].

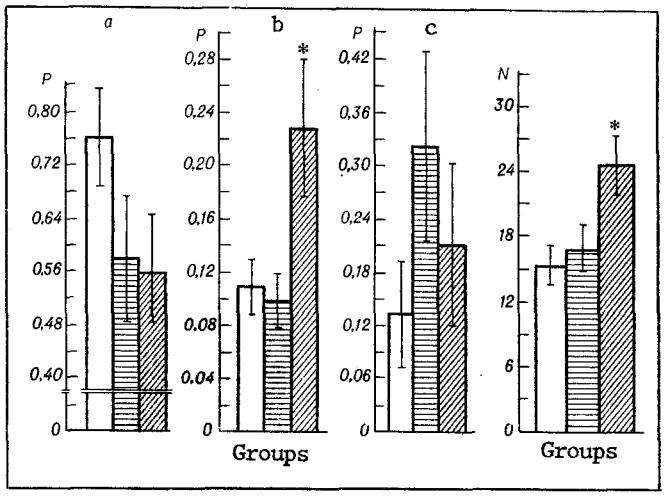

Fig. 2 Fig. 3

Fig. 2. Probability of finding rats in different parts of passive avoidance chamber. a) Light compartment, b) entrance into dark compartment, c) dark compartment. Unshaded column — intact group, horizontal shading — control group, oblique shading — experimental group. *$p < 0.05$.

Fig. 3. Number of times of passing photoelectric transducers in region of entrance into dark compartment.

TABLE 2. Latent Periods of Visiting and Relative Percentage of Animals Found in Dark Compartment

Group	Latent period, sec	Relative percentage
Intact (n = 17)	37,8±8,1	29,4
Control (n = 13)	70±41,2	53,8
Experimental (n = 16)	121,3±73,5	43,8

EXPERIMENTAL RESULTS

At the end of electrophoresis, 12 or 13 stained protein fractions were counted in the gel columns (Fig. 1). Application of 10^{-3} M serotonin solution to the occipital region of the rats' cerebral cortex led to a reproducible and significant ($p < 0.05$) change in the content of fraction No. 6 (Fig. 1, Table 1). The predominant type of change was a decrease in the content of this fraction (in 10 of 12 experiments). Meanwhile application of a 10^{-3} M noradrenalin solution caused no significant changes in the content of fraction No. 6 (Table 1) or of any other fraction.

When the passive avoidance model was used, intraventricular injection of immunoglobulins to fraction No. 6 caused a significant ($p < 0.05$) increase in the probability of finding the animals (Fig. 2) and the number of times of passing the photoelectric transducers (Fig. 3) in the region of the entrance to the dark compartment during the testing sessions compared with the control and intact animals. The probabilities of finding the rats in the light and dark compartments (Fig. 2), the latent periods of their visits, and the relative percentages of animals visiting the dark compartment (Table 2) did not differ significantly between the groups. Visually, in animals of the experimental group, more horizontal movements and vertical rearings were observed.

The results indicate selective involvement of serotonin in the regulation of metabolism of individual water-soluble protein fractions in the cerebral cortex. This involvement is perhaps mediated through corresponding receptors and secondary messengers and is realized at the level of transcription—translation processes. In particular, the predominant inhibitory action of serotonin on the content of fraction No. 6 which was found is in agreement with the results of investigations [7] showing a decrease in the total protein content in neurons of the hippocampus and motor cortex in rats under

the influence of an excess of serotonin, and also with the results of experiments [1] showing inhibition of protein synthesis in surviving hippocampal slices in the presence of serotonin. Meanwhile the possibility of posttranslational modification of proteins, leading to a change in their charge and to their redistribution in the process of electrophoretic fractionation, cannot be ruled out. The results of the behavioral experiments indicate, in our view, an increase in the investigative activity of the animals under the influence of immunoglobulins to fraction No. 6. This conclusion is based on the fact that the number of times of passing the photoelectric transducers was increased in the case of animals of the experimental group, leading to increased probability of their discovery in the region of the entrance into the dark compartment. Although the role of the proteins of fraction No. 6 in the activity of the nerve cells is not yet clear. the character of the changes in the rats' behavior under the influence of immunoglobulins to this fraction is in agreement with data obtained by other workers [4, 15], who found that the serotoninergic system is involved in the organization of investigative behavior.

LITERATURE CITED

1. V. M. Getsova, R. York, and W. Wetzel, Zh. Vyssh. Nerv. Deyat., **30**, 988 (1980).
2. E. A. Gromova, V. G. Tsyganova, and A. R. Chubakov, Proceedings of the 9th All-Union Conference on Biochemistry of the Nervous System [in Russian], Moscow (1988).
3. Enzyme Immunoassay [in Russian], Moscow (1988).
4. Kh. Yu. Ismailova, G. G. Gasanov, T. P. Semenova, et al., Zh. Vyssh. Nerv. Deyat., **39**, 548 (1989).
5. R. I. Kruglikov, Neurochemical Mechanisms of Learning and Memory [in Russian], Moscow (1981).
6. G. V. Lakin, Biometrics [in Russian], Moscow (1990).
7. V. I. Mats, O. L. Segal, and R. I. Kruglikov, Izv. Akad. Nauk SSSR, Ser. Biol., No. 2, 282 (1989).
8. L. A. Osterman, Investigation of Biological Macromolecules by Isoelectric Focusing, Immunoelectrophoresis, and Radioisotope Methods [in Russian], Moscow (1983).
9. A. Ashkenazi, J. Ramachandran, and D. J. Capon, Nature, **340**, 146 (1989).
10. B. Davis, Ann. New York Acad. Sci., **121**, 404 (1964).
11. W. B. Essman and S. C. Essman, Behavioral Neurochemistry, New York (1977), p. 25.
12. M. B. Kennedy, Neural and Molecular Bases of Learning, Chichester (1987).
13. B. L. Roth and D.-M. Shuang, Life Sci., **41**, 1051 (1987).
14. V. E. Shashoua, Int. Rev. Neurobiol., **16**, 183 (1974).
15. C. H. Vanderwolf, Brain Res., **504**, 192 (1989).