4. Yu. D. Ignatov and Yu. N. Vasil'ev, Abstracts of Proceedings of an All-Union Conference on Reflex Therapy [in Russian], Leningrad (1984), pp. 27-28.
5. V. K. Reshetnyak, Progress in Science and Technology. Series: Physiology of Man and Animals [in Russian], Vol. 29, Moscow (1985), pp. 39-103.
6. J. S. Cheng, S. P. Ou, and X. P. He, Chung Kuo Yao Li Hsueh Por., 3, 154 (1982).
7. F. Dittmar, Dtsch. Z. Akupunktur, 3, 95 (1977).
8. B. Kaada, Adv. Pain Res. Ther., 1, 733 (1976).
9. G. Pauser, Dtsch. Z. Akupunktur, 2, 31 (1977).
10. B. Pomeranz and R. Cheng, Exp. Neurol., 64, 327 (1979).
11. R. S. Snider and W. T. Neimer, A Stereotaxic Atlas of the Cat Brain, Chicago (1965).
12. C. Takeshige, Y. Kamada, and T. Hisamitsu, Acupuncture Electrother. Res., 6, 57 (1981).
13. K. Toda, M. Ishioka, A. Iriki, and H. Suda, Exp. Neurol., 64, 704 (1979).
14. R. Umlauf, Stsch. Z. Akupunktur, 4, 121 (1982).
15. W. Zhang, B. Zhang, W. Yu, and J. Xu, Acupuncture Res., 9, 51 (1984).
16. L. Zhou, J. Hu, Z. Guan, et al., Acupuncture Res., 9, 33 (1984).

EFFECT OF THYMALIN ON PROTEIN SYNTHESIS IN THE BRAIN AND ON CONDITIONED-REFLEX ACTIVITY OF THE OFFSPRING OF NEUROSENSITIZED RATS

N. A. Trekova

UDC 616.831-008.9-056.43-055.52-055.2-092.9-07.
616:831-008.939.6-053.2-02:615.276.4

KEY WORDS: neurosensitization; offspring; thymalin; brain proteins; defensive conditioned reflex

Experimental studies in recent years have revealed a number of important aspects of the pathogenesis of congenital diseases of the CNS connected with neuroimmunologic conflict between mother and fetus [6]. Besides a progressive neuroautoimmune process in the offspring of neurosensitized female rats, marked changes in protein synthesis in the CNS [7] and disturbance of ability to learn [2] have been found. A very important problem is that of the action of immunoregulators and, in particular, of the Soviet preparation thymalin, which has been successfully used in recent times in the combined treatment of several of the diseases mentioned above [9], on the brain. Restoration of ability to form an instrumental reflex to food in rats after injection of thymus extract was reported in [3]. The remaining experimental studies have been devoted mainly to the effect of thymalin on the immune system and on processes of regeneration [5, 8].

The aim of this investigation was to study the characteristics of protein synthesis in various parts of the brain and also the formation and preservation of a conditioned passive avoidance reflex (CPAR) in the offspring of intact and neurosensitized rats after treatment with thymalin.

EXPERIMENTAL METHOD

Four groups of animals aged 1.5-2 months (altogether 134 noninbred albino rats) were used in the experiments: group 1 consisted of offspring of intact mothers (control); group 2 was the offspring of intact mothers receiving thymalin at the age of 2 weeks (0.3 mg/100 g intramuscularly, daily for 5 days); group 3 consisted of offspring of mothers sensitized 2 weeks before mating with a 20% saline extract of cerebral cortex of allogeneic brain (0.5 ml intraperitoneally, three times at intervals of 1 day); group 4 was the offspring of neurosensitized mothers receiving a course of thymalin treatment at the age of 2 weeks.

In the study of protein synthesis (40 rats) ^{3}H-leucine (200 μCi per animal, intraperitoneally) was used as the precursor. Rats were decapitated 1 h later and four brain structures

Department of Psychiatric Pathobiology, Moscow Research Institute of Psychiatry, Ministry of Health of the RSFSR. (Presented by Academician of the Academy of Medical Sciences of the USSR G. N. Kryzhanovskii.) Translated from Byulleten' Éksperimental'noi Biologii I Meditsiny, Vol. 104, No. 11, pp. 535-537, November, 1987. Original article submitted May 12, 1986.

 0007-4888/87/0011-1512$12.50

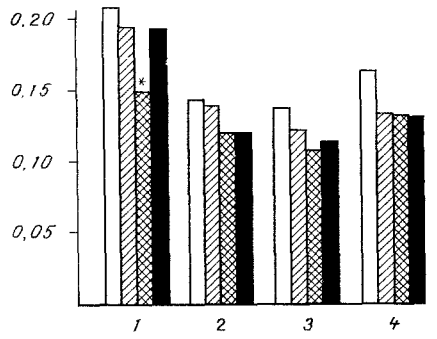

Fig. 1

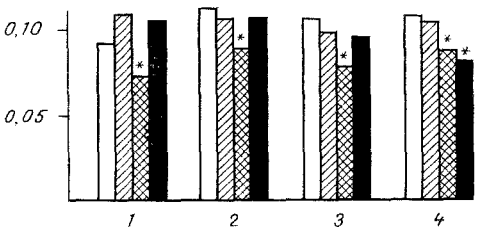

Fig. 2

Fig. 1. Effect of thymalin on incorporation of ^{3}H-leucine into water-insoluble brain proteins of offspring of intact and neurosensitized rats. Ordinate, relative specific activity. Unshaded columns — offspring of intact mothers (group 1, control); obliquely shaded columns — the same after treatment with thymalin (group 2); cross-hatched columns — offspring of neurosensitized mothers (group 3); black columns — the same after treatment with thymalin (group 4). 1) Cerebral cortex; 2) hippocampus; 3) basal ganglia; 4) brain stem. *$P < 0.05$ compared with control.

Fig. 2. Effect of thymalin on incorporation of ^{3}H-leucine into water-soluble brain proteins of offspring of intact and neurosensitized rats. Legend as to Fig. 1

were removed on ice, and homogenized in distilled water. The homogenates were centrifuged and proteins were precipitated with 10% TCA separately in the aqueous supernatants (the water-soluble protein fraction — WSP) and the residues (water-insoluble proteins — WIP). Precipitated proteins were treated by the usual method with extraction of lipids and subjected to alkaline hydrolysis in 0.5 N NaOH with heating. Radioactivity of the proteins and TCA-supernatants was determined in toluene-ethanol scintillator on a RackBeta liquid scintillation counter (LKB, Sweden). The relative specific activity — the ratio of radioactivity of proteins to activity of the corresponding TCA-supernatants — was calculated.

A CPAR (94 rats) was formed by the method in [12]. The maximal length of stay in the lit compartment of the apparatus when testing preservation of CPAR was 600 sec. The difference between the time spent in the lit compartment when testing preservation of CPAR and the time in the initial experiment (Δt) was used as the criterion of preservation of the formed reflex.

The results were subjected to statistical analysis by the Wilcoxon—Mann—Whitney and Student tests.

EXPERIMENTAL RESULTS

Injection of thymalin into the offspring of intact rats (group 2) caused no significant changes in protein synthesis in the CNS compared with the control (Figs. 1 and 2). A tendency was observed for incorporation of the label into WIP of the brain-stem structures to decrease and for radioactivity of WSP in the cerebral cortex to increase. The time of formation of CPAR in these rats did not differ from the control, but values relating to preservation of the reflex were significantly higher (Table 1).

In the animals of group 3 significant inhibition of synthesis of WSP in all parts of the brain studied and also of synthesis of WIP in the cortex was observed compared with intact rats. Reduction of incorporation of ^{3}H-leucine into WIP of the remaining structures was characterized as a marked tendency ($p > 0.05$). In these rats the parameters of preservation of CPAR were significantly lower (by more than half compared with the control) whereas the time of formation of the reflex was virtually unchanged.

Changes in protein synthesis in the animals of group 4 were varied in direction. Incorporation of the label into WIP of the hippocampus and basal ganglia and also into WIP and WSP of the brain-stem structures was just as low as in the rats of group 3. Meanwhile, compared with the animals of group 3, in those of group 4 significant activation of synthesis of both

TABLE 1. Effect of Thymalin on Formation and Preservation of CPAR in Offspring of Intact and Neurosensitized Female Rats (M ± m)

Group of animals	Duration of stay in lit compartment, sec		Parameter of preservation of CPAR (Δt) sec
	initial experiment	24 h later	
1 (control)	7,4±0,9	359,8±59,2	350,1±55,9
2	7,0±0,7	522,4±36,7*	506,5±36,8*
3	5,2±0,6	174,4±52,4*	169,1±52,9*
4	23,8±8,2***	436,1±101,2**	412,2±47,1**

Legend. *$p < 0.05$ compared with control, **$p < 0.05$ compared with group 3.

protein fractions was observed in the cortex and of WSP in the basal ganglia ($p < 0.05$). These parameters were close in value to the controls. The time of formation of CPAR in the animals of group 4 was substantially increased, and the parameters of preservation of CPAR were significantly higher in value than in the rats of group 3, and a little higher than the control level.

The experimental results showed that injection of thymalin into intact animals in the early stages of development does not induce changes in protein synthesis in the brain. Meanwhile, a substantial improvement of the parameters of preservation of CPAR was observed in such rats. Inhibition of protein synthesis in the CNS was found in the offspring of neurosensitized rats. Under these circumstances protein synthesis was most seriously affected in the cerebral cortex — the same as the part used for sensitization, and also of WSP in other brain structures. These findings differ to some degree from those obtained by the writers previously [7], possibly because of technical differences and the use of different precursors of protein synthesis [11]. Preservation of CPAR also was considerably impaired in these animals, evidence of definite disturbances of memory mechanisms. A course of thymalin given to the offspring of the neurosensitized rats caused virtually complete recovery of the depressed parameters of synthesis of both protein fractions in the cortex and of WSP in the basal ganglia and hippocampus. Meanwhile the parameters of CPAR preservation were restored to normal.

The definite parallel observed in the direction of changes in protein synthesis (WIP and WSP in the cortex, WSP in the hippocampus) and the parameters of CPAR preservation in the experimental groups will be noted, and in conjunction with data in the literature [4], it may be evidence that the processes of consolidation are dependent on the intensity of protein synthesis in the CNS.

If the probable mechanisms of normalization of the metabolic and functional parameters of CNS activity in the offspring of the neuroimmunized mothers after a course of thymalin treatment are examined, the following hypothesis can be put forward. First, thyamlin may suppress the autoimmune process in the early stages of its development, as a result of which autoimmune injury to the CNS is "registered". Second, considering the existing data on screening of T cells from the action of antibrain sera by thymalin [1] and the common antigenic features of T cells and the brain [10], the possibility of a direct protective action of thymalin on the brain cannot be ruled out, i.e., screening of the CNS from the pathogenic action of antibrain autoantibodies.

The investigation thus showed that a course of thymalin injections given to the offspring of neurosensitized mother rats at the age of 2 weeks prevents these animals from developing disturbances of synthesis of water-soluble proteins in the brain and of their conditioned-reflex activity.

LITERATURE CITED

1. G. A. Belokrylov and B. V. Popov, Immunology, No. 1, 30 (1985).
2. P. B. Kazakova, G. F. Konokotina, and V. N. Provodina, Clinical Aspects of Congenital Brain Pathology and the Pathogenesis of Nervous and Mental Disorders [in Russian], Moscow (1979), pp. 74-87.

3. I. M. Kiselev and G. A. Belokrylov, Byull. Éksp. Biol. Med., 94, No. 10, 15 (1982).
4. R. I. Kruglikov, Neurochemical Basis of Learning and Memory [in Russian], Moscow (1981).
5. B. N. Safronov, V. G. Morozov, V. Kh. Khavinson, and G. A. Belokrylov, Fiziol. Cheloveka, 10, No. 2, 229 (1984).
6. S. F. Semenov and K. A. Semenova, Immunobiological Basis of the Pathogenesis of Nervous and Mental Diseases [in Russian], Tashkent (1984).
7. N. A. Trekova, "Function of the blood-brain barrier and protein synthesis in different parts of the brain of rats undergoing neuroimmunization, and of their offspring," Author's Abstract of Dissertation for the Degree of Candidate of Medical Sciences, Moscow (1983).
8. V. Kh. Khavinson and V. G. Morozov, Uven.-Med. Zh., No. 5, 37 (1982).
9. N. T. Yakovleva, I. P. Mamonova, and I. P. Lazarenko, Immunopathology of Nervous and Mental Diseases [in Russian], Moscow (1983), pp. 154-155.
10. C. Bron, J. J. Pahud, and D. Sauser, J. Immunol. Meth., 12, No. 1-2, 39 (1976).
11. M. Hershkovitz, J. E. Wilson, and E. Glassman, J. Neurochem., 25, No. 11, 687 (1975).
12. M. E. Jarvik and R. Kopp, Psychol. Rep., 21, No. 8, 221 (1967).

MECHANISMS OF THE STIMULATING EFFECT OF ANTIBRAIN ANTIBODIES ON Ca^{++} CURRENTS IN THE NEURON MEMBRANE

E. I. Solntseva, A. L. Pozdnyakova, V. Savić, J. Horvat, and B. Janković

UDC 612.822.2.014:576.314].085

KEY WORDS: antibrain antibodies; Ca^{++} currents; molluscan neurons

The study of the mechanisms of interaction of antibrain antibodies with antigens of the neuron membrane is an urgent problem in normal and pathological physiology. It was shown previously that antibodies to the microsomal fraction and to synaptic vesicles of the rat brain can potentiate the Ca^{++}-component of action potentials of snail neurons [6, 7].

The aim of this investigation was to study the mechanisms of this effect of antibodies in voltage clamp experiments with recording of Ca^{++}-currents.

EXPERIMENTAL METHOD

Experiments were carried out on isolated unidentified neurons of *Helix pomatia* by the voltage clamp method, using two microelectrodes. Before isolation of the neurons, the nerve ganglia were incubated in an 0.5% solution of trypsin, made up in standard Ringer's solution for snails, at 30°C for 20-30 min. The isolated neurons were placed in a perfusion chamber with a volume of 0.14 ml. The rate of flow of the solution in the chamber was 0.6 ml/min. To record Ca^{++}-currents the standard Ringer's solution in the chamber was replaced by a sodium-free solution containing K^+-channel blockers: 10 mM $CaCl_2$, 4 mM KCl, 4 mM $MgCl_2$, 95 mM tetraammonium bromide, 5 mM 4-aminopyridine, 5 mM Tris-HCl (pH 7.6). To study the effect of Cd^{++} ions on Ca^{++}-currents, $CdCl_2$ was added to the above solution in a concentration of $5 \cdot 10^{-6}$ to $5 \cdot 10^{-4}$ M.

The microelectrodes were filled with 2 M KCl and their resistance was 5-10 MΩ. A standard apparatus (Nihon Kohden, Japan) was used to clamp the voltage on the membrane and record ionic currents. The preparation of transition processes during a stepwise change of potential in these experiments was 0.3-1 msec.

The microsomal fraction of the rat brain and antibodies to this antigen were obtained by methods described previously [5, 8]. In the control experiments, blood serum γ-globulins of an unimmunized rabbit were used. Antibodies or control γ-globulins were diluted in the solution used to record Ca^{++}-currents, either without Cd^{++} ions or with the addition of $2 \cdot 10^{-5}$ M Cd^{++}, depending on the experimental conditions. A glass micropipet with broken off tip, lo-

Laboratory of Functional Synaptology, Brain Institute, All-Union Mental Health Research Center, Academy of Medical Sciences of the USSR, Moscow. Immunologic Research Center, Belgrade, Yugoslavia. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Snezhnevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 11, pp. 537-539, November, 1987. Original article submitted March 5, 1986.

