total whole brain γγ-enolase activity did not exceed 8.6% (which corresponds to inhibition of about 35-40% of all enolase activity in the neurons). However, the conventional nature of the assumption that antiserum is uniformly distributed throughout the mass of the brain will be evident. There is no doubt that local inhibition of γγ-enolase is much more marked. This hypothesis is in full agreement with the fact that ethanol consumption is reduced in animals receiving immune serum. The neuronal enolase isozyme evidently participates in the mechanisms of formation of addiction to ethanol. The molecular mechanism of the effect of γγ-antiserum on ethanol consumption is not clear. It can be tentatively suggested that inhibition of glycolytic neurospecific γγ-isozyme lowers the intensity of energy metabolism in neurons. It is evident that γγ-antiserum has no direct effect on the enzyme systems of glycolysis in the liver, in which up to 95% of ethanol entering the body is oxidized [5, 7]. The inhibitory action of γγ-antiserum on ethanol consumption is therefore evidently determined by the central effects caused by reduction of the energy supply to the brain neurons.

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CHARACTERISTICS OF MEMORY IN MRL/1 MICE AND EFFECT OF THYMIC PEPTIDES ON IT

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UDC 616.72-002.77-039-092.9-092:612.821.2.06:612.438.4

KEY WORDS: long-term memory; autoimmune process; thymic peptides.

Much progress has been made in recent years in our understanding of the immunopathogenesis of rheumatic diseases [11]. Nevertheless their onset after stressful situations, the high frequency of placebo effects, and the absence of any parallel trend between clinical manifestations and immunologic parameters cannot be explained by the autoimmune theory of development. It is therefore important to study the physiological mechanisms maintaining the immune control of homeostasis, i.e., the suprasystemic mechanisms of regulation of immunity [5, 12], in particular, from the standpoint of N. P. Bekhtereva's concept of the stable pathological state of the brain on the matrix of long-term memory as its basis [2].

We shall examine one of the commonest forms of rheumatic disease, namely rheumatoid arthritis (RA), as a disease of adaptation with primary disturbance of the suprasystemic mechanisms of regulation of the immune control of homeostasis, realized clinically as RA by virtue of a genetic predisposition toward the development of an autoimmune lesion of connective tissue and, in particular, of joints [9]. With the foregoing facts in mind, it was

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(Presented by Academician of the Academy of Medical Sciences of the USSR V. A. Nasonova.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 7, No. 2, pp. 199-201, February, 1989. Original article submitted September 10, 1987.

0007-4888/89/0002-0212$12.50

decided to study the characteristics of memory in MRL/1 mice, which are an experimental model of RA [8]. The high clinical efficacy of a polypeptide preparation from the thymus, namely thymalin [8], and the role of oligopeptides in the mechanism of memory [1], and the role of thymectomy in MRL/1 mice in the realization of their predisposition to autoimmune disease [13], have been reported. In our view some peptides from the thymus are humoral factors of the "antisystem" (to use Kryzhanovskii's words [6]) of the immune control of homeostasis, and the thymus is an important organ of that system. These considerations formed the basis for the present investigation.

EXPERIMENTAL METHOD

Experiments were carried out on 25 MRL/1 mice and 25 CBA mice (control) weighing 25-34 g. To evaluate differences in the formation and long-term storage of an acquired skill, the method of active and passive avoidance of electrical stimulation [10] was used. The animals were placed in a specially constructed apparatus. An acoustic stimulus was applied for 5 sec, followed by an electric current for 10 sec (1.5 times the threshold strength for vocalization), and when the mice jumped on to a special platform the current was switched off. During training and subsequent testing of the degree of preservation of the engram of the acquired skill of avoidance, 10 combinations of the conditioned and unconditioned stimuli were applied with an interval of 10 sec between them. The number of times the mice jumped on to the platform during the action of the conditioned stimulus (active avoidance reaction – AAR) or of the unconditioned stimulus (passive avoidance reaction – PAR) was counted.

Mice of both lines were randomized into two groups by a simple method using random number tables [4]: thymalin was injected intraperitoneally into the first group in a dose of 0.2 μg in 0,2 ml of 0.14 M NaCl, while the second group received 0.2 ml of solvent only.

The effect of a single injection of thymalin given 1 h before the beginning of training on learning was judged by the number of correct responses (jumping on to the platform) on the 1st and 3rd day of training. The effect of thymalin on consolidation of the engram of long-term memory was assessed by the number of correct responses during testing the acquired skill 1 and 3 weeks after completion of a 6-day course of daily training. For this purpose thymalin was injected in a single dose intraperitoneally immediately after the end of training on the 6th day, i.e., at the final stage of consolidation of the engram of the acquired skill. The numerical data were subjected to statistical analysis by the signs test (p_1) and the chi-square test [7].

EXPERIMENTAL RESULTS

It will be clear from Table 1 that the MRL/1 mice, unlike the controls, had difficulty in forming engrams of the acquired skill: by the 3rd day of training their PAR was 32% lower ($p_2 < 0.002$). In the final stage of consolidation by the 6th day of training differences between the experimental and control groups of mice had disappeared, with respect to both AAR and PAR. This is probably the basis for a whole series of psychophysiological features peculiar to mice of this line, and determining the efficiency of their memory processes [3]. Their genetic determination is in agreement with disturbance of immunologic memory in MRL/1 mice [15].

In the mice of the experimental group the storage of the engram of the acquired skill was disturbed: after 1 week, extinction of the skill by 44% was observed with respect to AAR. In mice of the control line there was no significant time course of the parameters AAR and PAR. Another 2 weeks later, in the group of control mice the number of correct response in AAR was increased by 55%. In MRL/1 mice, however, the acquired skill was extinguished by two-thirds in AAR.

In CBA mice thymalin adversely affected the initial period of formation of the skill, reducing the number of correct jumps in AAR sixfold on the 1st day ($p_2 = 0.05$). Worsening ($p_2 < 0.002$) of the PAR parameter and complete absence of correct responses were observed. Thymalin had no significant effect on the parameters of CBA mice on the 3rd day of training. Injection of thymalin into MRL/1 mice caused worsening of the values of PAR by 34% ($p_2 < 0.002$). Compared with CBA mice receiving thymalin, there was a more than twofold difference with respect not only to PAR, but also to AAR ($p_2 < 0.002$). Thymalin thus inhibits the formation of long-term memory independently of the line of animals.

Thymalin has a positive effect on consolidation of engrams of an acquired skill. Its injection into CBA mice led to an increase of 57% in the value of AAR during 1 week, and in

TABLE 1. Parameters of Formation and Long-Term Storage of Engram of Acquired Skill in MRL/1 and CBA Mice and Effect of Thymalin on Them

Group of animals	Parameter	Period of formation of engram of skill				Period of storage of engram of skill							
		1st day		3rd day		6th day		13th day		6th day		23rd day	
		AAR	PAR	AAR	PAR	AAR	PAR	AAR	PAR	AAR	PAR	AAR	PAR
CBA	TCR	6	64*	21	109*	29	98*	30	103*	22	70*	34*	67*
	TSP	130	130	130	130	110	110	110	110	70	70	70	70
	p_2			<0,002	<0,002			>0,05	>0,05			<0,002	>0,05
MRL/1	TCR	2	34	13	74*	18	101*	10	100*	18	101*	6	97*
	TSP	130	130	130	130	110	110	110	110	110	110	110	110
	p_2			<0,002	<0,002			<0,01	>0,05			<0,01	>0,05
CBA with thymalin	TCR	1	64*	20	104*	21	70*	33*	70*	14	60*	23*	58*
	TSP	120	120	120	120	70	70	70	70	60	60	60	60
	p_2			<0,002	<0,002			<0,05	>1,00			>0,05	>0,05
MRL/1 with thymalin	TCP	0	13	9	49	13	97*	23	118	13	81*	13	83*
	TSP	120	120	120	120	130	130	130	130	100	100	100	110
	p_2			<0,002	<0,002			>0,05	<0,002			>0,05	>0,05

Legend. TCR) Total number of correct responses in group, TSP) total number of stimuli presented in group. Asterisk marks data for which $p_1 \leq 0.05$.

MRL/1 mice an increase of 22% in the value of PAR. Comparison of the time course of AAR, calculated per mouse in the MRL/1 groups without injection of thymalin (reduction of the parameter by 44%) and with its injection revealed the positive effect of the peptide on the consolidation process as early as 1 week after training ($p_2 < 0.002$). Injection of thymalin protected the engram of the acquired skill in MRL/1 mice: the difference in the time course during 3 weeks compared with intact MRL/1 mice is significant ($p_2 < 0.002$). Meanwhile the level of training remained lower in MRL/1 mice, despite the use of thymalin, than in CBA mice, with respect both to AAR ($p_2 < 0.002$) and to PAR ($p_2 < 0.01$).

The results suggest that slowing of formation of engrams of acquired skills and disturbance of their storage is one of the factors responsible for the lower adaptability of MRL/1 mice to changing external and internal environmental conditions, thereby facilitating realization of their genetic predisposition to an autoimmune process. The essential role of the thymus in determination of the state of higher nervous activity of animals and man must be assumed.

The authors are grateful to A. P. Dolzhenko and O. M. Samoilovich, assistant professors in the Department of Pharmacology, Donetsk Medical Institute, for help with setting up the investigation.

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EFFECT OF THE N-TERMINAL FRAGMENT OF SUBSTANCE P_{1-4} ON SOMATIC STRESS RESPONSE AND CATECHOLAMINE LEVELS IN RAT ADRENALS

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UDC 612.452.018.06:613.863].06:612.822.018:577.175.82

KEY WORDS: stress; substance P; catecholamines.

Investigations have shown [4-6] that the endogeneous neuropeptide substance P_{1-11} has an antistressor effect. Its normalizing, regulatory action has been observed on certain functional changes induced by chronic stress. According to Selye [8], a principal role in the development of the stress response of the body is played by catecholamines (CA) and, in particular, by CA of the adrenal medulla. Substance P also has been shown to be present in adrenal tissue, in the so-called S-ergic cells [3]. Substance P, released along with CA, can participate both in the regulation of certain physiological processes and in the maintenance of homeostasis during the development of stress [4, 6]. Oehme suggested that the main contribution to the antistressor effect of substance P belongs to the N-terminal end of the molecule of this neuropeptide [5].

This paper gives the results of a study of the somatic manifestations of the stress reaction in its dynamic course, and changes in the CA levels in the adrenals after preliminary injection of substance P_{1-4}.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats weighing 200 ± 20 g, immobilized in special frames which fixed the cervical and lumbar regions [2]. Preliminary experiments were carried out to study different stages of development of the general adaptation syndrome (GAS) under the specific conditions of this model of stress. The effect of substance P_{1-4} on the stage of alarm (immobilization for 1 h) and the stage of exhaustion (48 h) was investigated.

After decapitation of the animals the lymphoid organs (thymus and spleen) and the adrenals were weighed and the state of the gastric mucosa determined. The concentrations of adrenalin (A) and noradrenalin (NA) also were determined spectrofluorometrically by Euler and Lisjajko's method in the adrenals. CA levels in the adrenocytes and noradrenocytes of the adrenal medulla were studied by cytochemical electron microscopy, using the JEM-100B electron microscope ("Jeol") and the method of Tranzer and co-workers, in our own modification [1, 9], suitable for estimating the CA concentration in chromaffin granules.

The N-terminal fragment of substance P_{1-4} (Arg-Pro-Lys-Pro) was injected intraperitoneally once a day in a dose of 100 μg/kg; to study the stage of alarm it was injected 3 times preliminarily and once immediately before the experiment; when the stage of exhaustion was studied it was given twice preliminarily and twice during stress.

The results were subjected to statistical analysis by Student's t test.

Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow. Institute of Physiologically Active Substances, Academy of Sciences of the GDR, Berlin. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 2, pp. 201-204, February, 1989. Original article submitted May 5, 1988.

0007-4888/89/0002-0215$12.50