Lithium hydroxybutyrate can thus exert an inhibitory action of GPEE irrespective of where the GPEE was created, on conduction of excitation provoked by the GPEE, and on structures receiving this excitation, or in other words, on all stages of the pathological system.

LITEPATURE CITED

1. E. I. Danilova, V. N. Grafova, and G. N. Kryzhanovskii, Byull. Éksp. Biol. Med., No. 6, 525 (1979).
2. T. A. Zamoshchina and A. S. Saratikov, in: New Data on the Pharmacology and Clinical Use of Lithium Salts [in Russian], Moscow (1984), pp. 15-22.
3. G. N. Kryzhanovskii, Determinant Structures in Pathology of the Nervous System [in Russian], Moscow (1980).
4. G. N. Kryzhanovskii, in: New Data on the Pharmacology and Clinical Use of Lithium Salts [in Russian], Moscow (1984), pp. 49-57.
5. B. I. Lyubimov, N. S. Tolmacheva, and R. U. Ostrovskaya, Farmakol. Toksikol., No. 3, 273 (1980).
6. R. U. Ostrovskaya and S. S. Trofimov, Byull. Éksp. Biol. Med., No. 2, 170 (1984).
7. A. S. Saratikov, in: Neurotransmitters and Mechanisms of Action of Neurotropic and Cardiovascular Drugs [in Russian], Moscow (1979), p. 19.
8. P. Ahlavalia and P. L. Singhal, Neuropsychopharmacology, 20, 483 (1981).
9. G. Boulard, J. Guérin, G. Scontrini, et al., Agressologie, 237, 17 (1982).
10. V. B. Brooks, D. R. Curtis, and J. C. Eccles, J. Physiol. (London), 135, 655 (1957).
11. H. Laborit, J. M. Jouany, J. Gérard, et al., Presse Méd., 68, 1867 (1960).
12. A. Pomeroy and M. J. Rand, Aust. N. Z. J. Psychiat., 5, 280 (1971).
13. E. Richelson, Science, 196, 1001 (1977).
14. S. Treiser and K. J. Kelar, Eur. J. Pharmacol., 57, 85 (1979).

EFFECT OF CHRONIC PSYCHOGENIC STRESS ON CHARACTERISTICS OF SOME RAT BRAIN SYNAPTIC MEMBRANE RECEPTORS

V. O. Nikuradze, V. V. Rozhanets, M. M. Kozlovskaya, and A. V. Val'dman

UDC 612.822.1-06:613.863

KEY WORDS: rats; chronic stress; depression of behavior; brain receptors.

To discover the cellular and molecular mechanisms of the chronic action of psychotropic drugs an important step is to study their effect on the neurochemical characteristics of the brain in animals with appropriate pathology of behavior, which can be corrected by the drugs concerned [2]. In the present investigation characteristics of α- and β-adrenoreceptors, and also of imipramine and benzodiazepine receptors in brain synaptic membranes of rats were studied after exposure to combined stress for 15 days by a modified Hecht's method [7].

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 180-200 g were used. Stress was induced in September-October, 1983, daily for 15 days by means of chronic reinforcement by painful electric shocks of a conditioned stimulus (flashes) according to a stochastic program, with probability of electric shocks of 0.5. The rats' behavior before and after the beginning of stress was assessed on the basis of their activity in an open field test, in a maze, and in a shuttle box [7].

On the 16th day the stressed and control (intact) animals were decapitated, the brain was quickly washed free from blood in 0.32 M sucrose solution, after which it was homogenized in the same solution (10% homogenate) in a glass homogenizer with Teflon pestle in 0.32 M su-

Research Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow.

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 9, pp. 275-277, September, 1985. Original article submitted June 15, 1985.

crose, made up in 0.05 M Tris-HCl with 1 mM EDTA, pH 7.4. The homogenate was centrifuged for 10 min at 1500g, the residue was discarded, and the supernatant was centrifuged for 20 min at 12,000g. The residue, consisting of the fraction of unpurified synaptosomes (fraction P_2) was suspended in 0.05 M Tris-HCl, pH 7.4 (5 ml of buffer to 1 g of original tissue), poured into polyethylene flasks, and kept at −20°C for 10 days. Before the experiment the suspension was thawed in cold water and centrifuged for 20 min at 12,000g; the residue was suspended in incubation medium, corresponding to the ^{3}H-ligand chosen, and used for radioreceptor studies not later than after 1.5 h. All operations from washing the brain until keeping the final suspension were carried out in the cold. Specific binding of ^{3}H-WB-4101 (30 Ci/mmole), ^{3}H-dihydroalprenolol (52 Ci/mmole), ^{3}H-flunitrazepam (84 Ci/mmole), and ^{3}H-imipramine (21 Ci/mmole; all these compounds were from Amersham Corporation, England) was carried out by known methods [4, 5, 10, 14] with certain modifications.

^{3}H-WB-4101: the incubation mixture contained the ^{3}H-ligand in concentrations of 0.15, 0.45, 0.75, 1.05, 1.35,and 1.65 nM, the displacing agent phentolamine in a concentration of 10^{-5} M, 0.5-0.6 mg protein of synaptic membranes, and 50 mM Tris-HCl, pH 7.4 and 10 mM $CaCl_2$ in 0.5 ml.

^{3}H-dihydroalprenolol: the incubation mixture contained the ^{3}H-ligand in concentrations of 0.45, 1.35, 2.25, 3.10, 3.90, and 4.5 nM, the displacing agent propranolol in a concentration of 10^{-5} M, 0.5-0.6 mg membrane protein, 50 mM Tris-HCl, pH 7.4, and 10 mM $MgCl_2$ in a volume of 0.5 ml.

^{3}H-flunitrazepam: the incubation mixture contained the ^{3}H-ligand in concentrations of 0.4, 1.2, 2.0, 2.8, 3.6, and 4.0 nM, the displacing agent diazepam in a concentration of 10^{-6} M, 0.4-0.5 mg of membrane protein in 0.5 ml of 50 mM Tris-HCl buffer, pH 7.4.

^{3}H-imipramine: the incubation mixture contained the ^{3}H-ligand in concentrations of 0.48, 0.80, 2.40, 5.60, and 7.20 nM, the displacing agent imipramine in a concentration of 10^{-5} M, 50 mM Tris-HCl containing 150 mM NaCl and 10 mM KCl, and 0.5-0.6 mg membrane protein in a volume of 0.5 ml.

Incubation was carried out for ^{3}H-imipramine and ^{3}H-flunitrazepam at 0-4°C, and for the remaining ligands at 25°C. In all cases incubation continued for 60 min, and was stopped by the addition of 3 ml of the corresponding cold buffer to the samples, followed by immediate filtration of the suspension through glass wool GF/B filters (Whatman, England). After washing twice with the same buffer (filtration and washing took not more than 15 sec) the filters were dried and extracted for 12 h in 5 ml of Bray's scintillator. After radioactivity had been counted on an SL-4000 scintillation counter (Kontron, France) Scatchard plots of the results were analyzed by HP-33E microcalculator (USA). The protein concentration in the samples was determined by the method in [13].

EXPERIMENTAL RESULTS

The situation of indeterminacy in the experiments with probable reinforcement by painful electric shocks was tolerated very badly by the rats. After the end of 15 days they developed a reactive state which could be defined as depression of behavior. Motor and investigative activity in the open field test and in a closed maze was depressed in these animals whereas the number of grooming acts was significantly increased (Table 1). The behavior of the animals in the shuttle box was unchanged (results not given). Incidentally, the behavioral disturbances observed were stable in character and persisted for at least 10 days [7].

The concentration (B_{max}) and affinity (K_d) of the imipramine receptors remained unchanged. The writers showed previously that exposure of the rats to similar stress increased the affinity of the ^{3}H-serotonin reuptake systems in rat brain synaptosomes [1]. There is now abundant and varied evidence to show that imipramine receptors may be centers of allosteric control of the rate of serotonin reuptake in nerve endings and platelets [6, 12]. Our own results did not contradict these conclusions, but were evidence that the affinity of the serotonin reuptake systems can change without any accompanying change in imipramine receptors.

The decrease in concentration of benzodiazepine receptors which we discovered is in good agreement with existing data showing that sufficiently intensive chronic stress in rats can lead to a reduction in density of both benzodiazepine and GABA receptors [8, 9] in the brain. On this basis it can be postulated that the depression of behavior which we observed can be interpreted as an enhanced degree of sedation, due to the chronically increased release of GABA and, perhaps, of certain hypothetical benzodiazepine ligands. The decrease in concentration of GABA- and benzodiazepine receptors is probably an adaptive response to this increase.

TABLE 1. Changes in Parameters of Rats' Behavior in Open Field Test and in Maze before and after Stress for 15 Days (M ± m)

Parameters studied during observation for 5 min	Before stress	The same animals after strees
Open field		
Number of animals	20	20
Latent periods	2,8±0,5	4,7±0,3*
Number of squares crossed	83,6±4,2	17,4±2,7*
Vertical activity	20,8±0,9	4,2±0,5*
Investigative activity (number of holes sniffed)	7,3±0,6	1,1±0,2*
Number of grooming acts	2,9±0,4	5,7±0,4*
Number of defecations	1,3±0,3	1,0±0,3
Maze		
Number of squares crossed in 2 min	20,4±1,1	5,7±0,8*

Legend. *P < 0.05.

TABLE 2. Effect of Chronic Induction of Neurosis in Rats on State of Some Brain Synaptic Membrane Receptors (M ± m)

Ligand	Parameter	Control	Neurosis
	K_d	3,12±0,2	5,2±0,5 †
^{3}H-dihydroalprenolol	B_{max}	101,6±3,4	96,6±7,2
	K_d	1,36±0,2	1,0±0,12
^{3}H-WB-4101	B_{max}	96,2±9,5	69,0±3,2*
	K_d	3,2±0,2	2,4±0,08*
^{3}H-flunitrazepam	B_{max}	927±36,5	825±26,2*
	K_d	7,56±1,7	6,9±0,24
^{3}H-imipramine	B_{max}	663±63	693±32

Legend. Mean results of four independent experiments are shown. Mean values of r for curves in Scatchard plots were 0.93. K_d) Dissociation constant (in nM). B_{max}) Concentration of specific binding sites (in femtomoles/mg protein). *P ≤ 0.05, †P ≤ 0.01.

Different types of chronic stress are known to increase the turnover and secretion of noradrenalin in the brain and at the periphery [3]. The writers showed previously that in this model of chronic stress the affinity of noradrenalin reuptake systems in the brain synaptosomes is significantly increased. The decrease in affinity of β-adrenoreceptors, demonstrated in the present investigation, like the decrease in B_{max} of the α_1-adrenoreceptors, may reflect a different aspect of the adaptive changes caused by increased noradrenalin secretion, namely the development of subsensitivity of these receptors.

Meanwhile it has been shown that reserpine-induced depression, which can be corrected by many antidepressants [11], is accompanied by an increase in density of β-adrenoreceptors; correction of behavior by drugs, moreover, is accompanied by normalization (lowering) of their level. We thus have no grounds for identifying the depression of behavior observed in this particular model with the depressive-like state of the animals observed, in particular, in reserpine depression. The results taken as a whole suggest that pathology of behavior in rats observed in the model used above may be classed as a depressive-like state rather than a neurosis-like state, and the model itself may be more appropriate for the study of the mechanisms of action of compounds with marked tranquilizing activity.

LITERATURE CITED

1. N. A. Avdulov, B. O. Nikuradze, A. V. Eremenko, et al., Byull. Éksp. Biol. Med., No. 8, 209 (1984).
2. A. V. Val'dman, in: Pharmacology of Antidepressants [in Russian], Moscow (1984), p. 5-87.
3. A. V. Kruglikov, Ya. A. Rosin, M. M. Gromakovskaya, et al., in: Proceedings of the 24th All-Union Conference on Problems in Higher Nervous Activity [in Russian], Moscow (1974), pp. 185-194.
4. V. V. Rozhanets, D. Yu. Rusakov, N. D. Danchev, and A. V. Val'dman, Byull. Éksp. Biol. Med., No. 7, 46 (1983).
5. V. V. Rozhanets, D. Yu. Rusakov, E. V. Tarasova, and A. V. Val'dman, Byull. Eksp. Biol. Med., No. 12, 52 (1983).
6. V. V. Rozhanets, in: Pharmacology of Antidepressants [in Russian], Moscow (1984), pp. 88-120.
7. N. M. Khonicheva and N. D. Danchev, Zh. Vyssh. Nerv. Deyat., (1984), (in press).
8. G. Biggio, M. C. Corda, A. Congas, et al., Brain Res., 229, 363 (1981).
9. C. Braestrup, M. Nielsen, E. B. Nielsen, and M. Lyon, Psychopharmacology, 65, 273 (1979).
10. D. B. Bylund and S. H. Snyder, Mol. Pharmacol., 12, 568 (1976).
11. S. M. Jancsar and B. E. Leonard, Biochem. Pharmacol., 32, 1569 (1983).
12. S. Z. Langer, C. Moret, R. Raisman, et al., Science, 210, 1133 (1980).
13. G. L. Peterson, Anal. Biochem., 272, 329 (1978).
14. B. Spyra and K. M. Pirke, Brain Res., 245, 179 (1982).

CHANGES IN RNA-POLYMERASE ACTIVITY IN HEART AND LIVER CELLS IN IMMOBILIZATION STRESS

M. P. Yavich and F. Z. Meerson

UDC 616.12+616.36]-008.931-092.9-02:613.863

KEY WORDS: stress; RNA-polymerase I; RNA-polymerase II; messenger RNA; ribosomal RNA.

In emotional-painful stress the rate of synthesis of total RNA and of proteins is drastically changed. Soon after the end of exposure to stress the rate of RNA and protein synthesis falls, but later it is restored and exceeds the control values [2]. To study the character of function of the protein-synthesizing system of cells under the influence of stress factors it is important to know how the rate of synthesis of different classes of RNA and, in particular, the rate of synthesis of messenger RNA (mRNA) and ribosomal RNA (rRNA) changes.

In the investigation described below activity of RNA-polymerase I, an enzyme transcribing ribosomal genes, and of RNA-polymerase II, an enzyme responsible for mRNA synthesis, was studied.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 180-200 g. The animals were fixed by the limbs in the supine position (immobilization stress) for 6 h. The stress syndrome which develops as a result of this procedure is accompanied by the development of gastric ulcers. The animals were investigated immediately after the end of exposure to stress, and again 12, 24, and 48 h later.

Activity of the RNA-polymerases was studied in isolated nuclei of the heart, liver, and spleen in a cell-free system. The nuclei were isolated by the method in [8] with certain modifications [3]. Activity of RNA-polymerases I and II was determined under optimal conditions for the action of each enzyme, and was tested with α-amantine. RNA-polymerase I is known

Laboratory of Pathophysiology of the Heart, Institute of General Pathology and Pathophysiology of the Heart, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Gorizontov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 9, pp. 277-279, September, 1985. Original article submitted May 21, 1984.

 0007-4888/85/0009-1170$09.50