CHANGES IN SYNAPTOSOMAL MEMBRANES IN RATS DEVELOPING CHRONIC NEUROSIS

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The dominant view that there are no pathomorphological changes in brain structure in neuroses has been revised in recent years [1]. Isolated observations have demonstrated microstructural cerebral changes in experimental neurosis: disintegration of the spinous apparatus, a decrease in the number of ribosomes, disturbance of metabolism of cortical synaptosomes, and changes in the microcirculation [3, 6, 8]. The aim of the present investigation was to assess the state of the neuronal membranes and of certain membrane processes

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 180-200 g were used. Neurosis was produced in the course of 15 days by the method in [5]. The process of transmitter reuptake was studied in the coarse synaptosomal fraction of rat brain by a radioisotope method. A suspension of synaptosomes (on average 2 mg protein/ml) was incubated for 3 min with different concentrations of labeled mediators at 37°C, with constant mixing in incubation medium of the composition described previously [1]. The volume of the sample was 1 ml. The reaction was stopped by filtration of 100 µl of incubation mixture on "Millipore" filters with pore diameter 0.45 µ, followed by washing with 2 ml of incubation medium. The filters were dried at room temperature and radioactivity of the samples determined by liquid scintillation counting on an SL-400 counter (Intertechnique, France) in Bray's scintillator. The results were analyzed by the double reciprocals method, by plotting Lineweater–Burk graphs. Changes in the state of the membranes were studied by the fluorescent probes method on a pure synaptosomal fraction of rat brain, subjected to cold and osmotic shock as recommended in [4]. 1-Anilinonaphthalene-8-sulfonate (OANS) was used as the probe: its fluorescence was excited at λ = 360 nm and recorded at λ = 480 nm on an Opton (West Germany) spectrofluorometer. The content of total lipids was determined on a photoelectric colorimeter by the reaction with phosphovanillin reagent after hydrolysis with concentrated sulfuric acid. Na,K-ATPase activity was determined by the method in [12] and protein by Lowry's method [11]. The results were processed by HP-33E electronic calculator (USA). All the radioactive reagents used, namely [^{3}H]dopamine (specific activity 1.48 GBq/mmole), [^{3}H]serotonin creatine-sulfate (specific activity 544 GBq/mmole), and [^{3}H]GABA (specific activity in GBq/mmole), were from Amersham Corporation (England); the ANS was from Sigma (USA). All reagents were analytically pure.

TABLE 1. Characteristics of Interaction of Fluorescent Probes with Synaptosomal Membranes of Control and Neurotic Animals

Animals	K_b, M^{-1}	N_{sp}	$K_b \cdot N_{sp}$, M^{-1}	K_d	F_{mol}	r
Control	$1{,}1 \cdot 10^4$	$1{,}7 \cdot 10^{-3}$	18,7	$5{,}1 \cdot 10^{-5}$	$4{,}3 \cdot 10^4$	0,99
Experimental (neurosis)	$2{,}1 \cdot 10^4$	$1{,}1 \cdot 10^{-3}$	23,5	$8{,}3 \cdot 10^{-5}$	$6 \cdot 10^4$	0,97

Legend. K_b) Binding constant of probes with membrane; N_{sp}) specific number of binding sites; $K_b \cdot N_{sp}$) total affinity of probes for membranes; K_d) dissociation constant of zone–membrane complex; F_{mol}) molar fluorescence; r) coefficient of correlation. Binding parameters of ANS calculated per milligram protein.

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TABLE 2. Characteristics of State of Transmitter Reuptake Systems in Rats after Development of Chronic Neurosis

Transmitter	Animals			
	control		experimental (neurosis)	
	K_M, M	V_{max}, M	K_M, M	V_{max}, M
Noradrenalin	$(6,8\pm2)\cdot10^{-7}$	$(1,6\pm0,8)\cdot10^{-12}$	$(2,7\pm0,3)\cdot10^{-7}$	$(1,4\pm0,2)\cdot10^{-12}$
Dopamine	$(9,1\pm0,4)\cdot10^{-9}$	$(7,4\pm0,1)\cdot10^{-12}$	$(4\pm1,1)\cdot10^{-8}$	$(7,1\pm0,4)\cdot10^{-12}$
Serotonin	$(8,6\pm0,1)\cdot10^{-8}$	$(4,1\pm0,1)\cdot10^{-12}$	$(3,3\pm0,2)\cdot10^{-8}$	$(3,9\pm0,3)\cdot10^{-12}$
GABA	$(6,4\pm0,2)\cdot10^{-6}$	$(4,2\pm1,1)\cdot10^{-9}$	$(2,7\pm0,9)\cdot10^{-6}$	$(4,5\pm1,3)\cdot10^{-9}$

Legend. K_m) Michaelis constant, V_{max}) maximal velocity of process. $P \leqslant 0.05$, $n = 4$.

TABLE 3. Changes in Na,K-ATPase Activity in Rats with Chronic Neurosis

Animals	Enzyme activity, μmoles P_i/mg protein/h
Control	12,625±1,4
Experimental (neurosis)	8,933±0,25

Legend. $n = 3$, $t = 2.68$, $R \leqslant 0.05$.

RESULTS AND DISCUSSION

After 15 days of exposure to conditions inducing neurosis the rats developed persistent behavioral changes, in the form of depression of investigative and motor activity (the "open field" test), and disturbance of their behavior in a T-maze.

The lipid content in the neuronal membranes of normal rats is 30-40% [7]. The present experiments showed that the protein/lipid ratio in synaptosomes of the control animals was 2.1:0.97 = 2.15, in good agreement with data in the literature [7]. Meanwhile, in synaptosomes obtained from neurotic rats, this ratio was 1.35:0.98 = 1.4; the quantity of lipid remained unchanged, but the protein content decreased (per gram original weight of the brain) compared with the control. This is most probably due to a decrease in the content of peripheral membrane proteins and ought to be reflected in the state of the membrane surface, and also in activity of integral proteins, especially transmitters and some enzymes.

The study of the state of the membrane surface by the fluorescent probes method (Table 1) showed that the quantum yield of fluorescence of ANS in synaptosomes of neurotic rats ($F_{mol} = 6\cdot10^4$) was higher than in the control ($F_{mol} = 4.3\cdot10^4$), possibly due to an increase in hydrophobicity of the membrane surface [4]. Further evidence in support of this is given by the increase in K_b of the probe and an increase in its total affinity for the membrane (which is proportional to K_b). The decrease in the number of binding sites for ANS with the membrane may be due to the fact that this probe binds both with lipid and with protein [4]. A decrease in the relative number of binding sites of the probe with the membrane surface therefore suggests that it was the content of peripheral proteins on the outer surface of the membrane that was reduced.

A study of the kinetics of monoamine reuptake by the synaptosomes (Table 2) showed that affinity of the carriers for substrate was increased (K_M neurosis < K_M control), whereas the number of carriers was unchanged (V_{max} neurosis = V_{max} control).

The change observed may be connected with increased accessibility of the active centers of the carriers for mediators secreted into the space on account of a decrease in the content of peripheral proteins on the outer surface of the neuronal membranes, facilitating reuptake and leading to a decrease in the content of transmitters in the space.

After chronic neurosis development, activity if Na,K-ATPase also fell (Table 3) or there was a decrease in the quantity of this enzyme, possibly on account of a change in the state of the membrane and the membrane lipids.

These experiments showed that in rats developing chronic neurosis considerable changes take place in the state of the neuronal membranes and activity of the membrane systems. De-

pression of monoaminergic and GABA transmission, according to the data described above, was probably due to a decrease in the content of transmitters in the presynaptic zone of the synaptic junctions on account of increased activity of the transmitter reuptake systems and, possibly, inhibition of the released transmitters, leading to a decrease in the content of the latter in the synaptic space.

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ACTION OF β-CARBOLINE DERIVATIVES ON EVOKED HIPPOCAMPAL UNIT ACTIVITY

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Specific benzodiazepine receptors (BDR), mediating their pharmacologic activity, have been found in the CNS of higher vertebrates, including man [8]. Originally it was considered that only pharmacologically active benzodiazepines (BD) interact with high affinity with BDR. More recently, however, several compounds with high affinity for BDR, but not exhibiting anticonvulsant or anxiolytic effects characteristic of BD at the clinical level, have been found. A special place among these compounds is occupied by β-carboline derivatives, which interact with high affinity with BDR, thereby giving rise to biochemical and behavioral changes opposite to the effects of BD [4]. It has been shown, for instance, that β-carboline derivatives such as 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM) and methyl-β-carboline-3-carboxylate possess marked ability to induce seizures in experimental animals. It has also been shown that the intensity of the convulsant activity of these derivatives correlates with the degree of occupancy of BDR by them *in vivo* [4].

The writers previously described the effect of BD on the evoked potential (EP) arising in area CA1 of the hippocampus in response to stimulation of Schaffer's collaterals [1]. The aim of the present investigation was to study the action of β-carboline derivatives in the same experimental system and to compare it with the effect of BD.

EXPERIMENTAL METHOD

Experiments were carried out on surviving hippocampal slices from Wistar rats aged 2-3 weeks, by the method described previously [1]. The synthesis of methyl ester of tetrahydro-β-carboline also was described previously [6]. The methyl ester of 1-phenyl-tetrahydro-β-carboline was synthesized as follows: 10 g tryptophan was dissolved in 50 ml of 1 M H_2SO_4,

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