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MEMBRANE ASPECTS OF CHRONIC STRESS: EFFECTS OF PSYCHOTROPIC DRUGS

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Summary. – *Chronic psychogenic stress for 15 days induces profound changes in synaptosomal membranes and membrane-related processes.* In vitro *the effects of psychotropic drugs on lipid peroxidation and uptake of neurotransmitters on membranes from stressed animals differ from control rats. Singled out groups of active and nonactive rats (forced swimming test) differ from each other by metabolic coefficients, basal and noradrenaline-stimulated activity of adenylate cyclase; those differences are season-independent. Subchronic administration of tranquilizers, nootropic drugs, tricyclic and atypical antidepressants after stress to active and nonactive rats showed that antidepressants and piracetam are potent in nonactive animals, while tranquilizers and piracetam are potent in active rats. Data suggests that membrane effects of psychotropic drugs should be evaluated on animals with model psychopathology and that initial reactivity of animals must be taken into account.*

Riassunto (Aspetti neurochimici dello stress cronico: effetti dei farmaci psicotropi). – *Lo stress psicogeno ripetuto (15 giorni) causa notevoli alterazioni delle membrane sinaptosomiali e dei meccanismi di transduzione. Gli effetti* in vitro *sulla perossidazione lipidica e sull'uptake dei neurotrasmettitori differiscono negli animali sottoposti allo stress dagli animali-controllo. I ratti sono stati suddivisi in «attivi» e «non attivi» in base alla risposta nel «forced swimming test». Indipendentemente dal periodo dell'anno questi gruppi presentano differenze nell'attività dell'adenilciclasi in condizioni di base e stimolata da noradrenalina. Gli antidepressivi triciclici e quelli «atipici» risultano efficaci nei ratti «non attivi», gli ansiolitici in quelli «attivi», mentre il piracetam è attivo in ambedue i gruppi. I dati indicano che la valutazione degli effetti dei farmaci psicotropi deve essere fatta tenendo conto della reattività iniziale degli animali e utilizzando appropriati modelli psicopatologici.*

Introduction

At different stages of experimental neurosis or emotional stress neurochemical processes in the brain are modified, these modifications are adaptive in nature and induce changes in the state of membranes, enzymes activity and receptors number.

Tranquilizers, antidepressants (AD's) and nootropic drugs are used for correction of neurosis and emotional stress. Usually, the effects of these drugs are evaluated on membranes from "control" healthy animals. On the other hand, psychotropic drugs, especially AD's, produce their psychotropic effect only in patients, but not in healthy volunteers. Moreover, there are some "atypical" psychotropic drugs which are not active in the traditional screening methods. Taking all this into account, we tried to find a model of experimental psychopathology in animals, to compare the state of membranes and membrane-related processes in control and "model" animals and to compare the effects of drugs on membranes from control animals to their effects on membranes from animals with induced psychopathology. As a model we used rats submitted to prolonged (15 days) psychogenic stress [1]. These animals exhibited decreased locomotor and exploratory activity ("open field" test) and impairment in T-maze performance [2].

Materials and methods

Crude synaptosomal fraction from rat brain (male albino mongrel rats, 180-200 g) was obtained by centrifugation of 10% homogenate (glass-tephlon homogenizer) in a media containing 0.32 M sucrose, 0.1 mM EDTA, 50 mM Tris-HCl buffer, pH = 7.4, at 1000 *g* for 10 min. Supernatant was collected and centrifuged at 11,000 *g* for 20 min. A pellet containing synaptosomes was resuspended in 50 mM Tris-HCl buffer, pH = 7.4. Pure fraction of synap-

tosomal membranes was obtained as described by Christian [3].

Chronic stress was performed according to slightly modified procedure of Hecht *et al.* [1] for 15 days. Rats were placed individually in chambers (15 × 19 × 20 cm) in darkness. The session lasted 14 min a day. During the session rats were exposed for 12 times to flashing light (1.4 Hz, 10 s each time) and in 6 exposures light was accompanied by electric foot shock (30 V, 5 s). The probability of shock was 50%. Shocks were given according to stochastic program in order to prevent formation of conditioned reflexes. Control group of animals was placed in the same chambers for 14 min daily in darkness before experimental group, but did not receive any light or electric stimulation.

Lipid peroxidation was evaluated by estimating malone dialdehyde (MDA) concentration after its reaction with thiobarbituric acid (TBA) [4]. For drugs, their ability to decrease the amount of MDA by 50% in presence of a mixture of $FeSO_4$ 10^{-5} M and ascorbic acid 10^{-4} M during 20 min of incubation at 37°C was registered. Influence of drugs on uptake of 5-HT, dopamine and GABA was evaluated with radioisotopes technique [5, 6]. Receptor binding studies were carried out using radioisotope technique and ^{3}H-DHA [7], ^{3}H-mianserine [8] as ligands. Adenylate cyclase activity was estimated with ^{32}P-α-ATP [9]. Binding sites for ^{3}H-DHA and activity of noradrenaline-stimulated adenylate cyclase were measured in whole brain without cerebellum, binding sites for ^{3}H-mianserine and activity of histamine-stimulated adenylate cyclase were measured in cerebellum, activity of dopamine-stimulated adenylate cyclase in brain cortex.

Metabolic parameters were measured for each single animal in plasma using clinical centrifuge analyser "Centrifichem-600" ("Baker", USA) and monotests "Baker" (USA), "Boehringer" (BRD). The metabolic coefficients measured characterize vulnerability of animals to stress-stimuli of any nature (endo- and exogenous). If levels of metabolites change slightly and in some cases nonsignificantly, metabolic coefficients will indicate significant changes if there are any.

Total lipid content was measured with "total lipids" test ("Chemapol", Chechoslovakia). Protein content was measured as described by Peterson [10].

Singling out groups of animals was performed by forced swimming test [11, 12]. Rats were placed in tanks with water (t° = 25°C) and for 10 min periods of active swimming and periods of immobilization (when rats were slightly moving their paws and tail in order to be able to breathe, but not swimming vigorously trying to escape the situation) were registered. Rats with time of immobilization less than 180 s were considered "active" and with time of immobilization of 300 s or more — "nonactive". Drugs were administered 24 hours after the last stress session for 7 days *per os*. Diazepam 1 mg/kg, piracetam 300 mg/kg, imipramine, mianserine and viloxazine 10 mg/kg.

Statistical and correlation analysis as well as other calculations were performed on PC-20 ("Commodore", USA). "Statgraf" and specially designed programs were used.

Results

Lipid peroxidation (LP) and stress. – As shown in Table 1, after chronic stress basal level of MDA in brain is increased, but stimulation with prooxidant was less effective than in control group.

Stress and adenylate cyclase activity. – Basal and dopamine-stimulated activity of adenylate cyclase in synaptosomal membranes from rat brain cortex are decreased (Table 1).

Table 1. – *Lipid peroxidation (LP) and adenylate cyclase activity in synaptosomes of rat brain cortex after chronic psychogenic stress*

	Control	Stress
Lipid peroxidation		
Basal	2.3 ± 0.3	3.5 ± 0.3 (*)
Stimulated	12.5 ± 0.4	10.2 ± 0.5 (*)
Adenylate cyclase activity		
Basal	117.0 ± 10.0	78.0 ± 8.0 (*)
DA-Stimulated	193.0 ± 15.0	131.0 ± 9.0 (*)

LP: mcM of MDA/g of lipid; adenylate cyclase activity: in pmol cAMP/mg of protein/min.
(*) p = 0.05.

Influence of drugs on membrane-related processes after chronic stress. – Our data shows that influence of drugs on membrane-related processes after chronic stress differs from their activity on membranes from control animals. For all studied drugs IC_{50} values in inhibition of lipid peroxidation test were several times higher in membranes from stressed rats compared to membranes from control ones (Table 2).

Table 2. – *Influence of some psychotropic drugs on LP in synaptosomal membranes from control and stressed rats*

Drug	IC_{50}, M	
	Control	Stress
Imipramine	6.5×10^{-6}	1.8×10^{-5}
DMI	2.7×10^{-6}	3.9×10^{-6}
Clorimipramine	6.5×10^{-6}	1.5×10^{-5}
Pirlindol	8.0×10^{-6}	2.7×10^{-5}
Chlorpromazine	7.8×10^{-6}	3.3×10^{-5}
Piracetam	2.7×10^{-5}	2.9×10^{-4}

IC_{50}: concentration of drugs that inhibits LP by 50%.

Table 3. – *Effect of antidepressants on uptake of ^{3}H-5-HT, ^{3}H-dopamine and ^{3}H-GABA by crude synaptosomal preparation from rat brain*

Drug	Conc. 10^{-6} M	Control			Stress		
		5-HT	DA	GABA	5-HT	DA	GABA
—	—	100 ± 9	100 ± 8.5	100 ± 8.2	373 ± 35(*)	130 ± 9.5(*)	143 ± 11(*)
Imipramine	5.0	39 ± 4 (**)	55 ± 4.9 (**)	82 ± 4.8 (**)	151 ± 11 (**)	68 ± 5.7 (**)	112 ± 7.6
	50.0	9 ± 1 (**)	28 ± 1.1 (**)	47 ± 3.9 (**)	30 ± 6 (**)	34 ± 6.4 (**)	68 ± 3.6
DMI	5.0	43 ± 4 (**)	49 ± 4.2 (**)	84 ± 5.8 (**)	168 ± 13 (**)	65 ± 6.6 (*)	116 ± 7.7 (**)
	50.0	11 ± 2 (**)	20 ± 0.5 (**)	52 ± 4.1 (**)	37 ± 11 (**)	25 ± 1.8 (*)	77 ± 4.9 (**)
Mianserine	5.0	49 ± 4 (**)	73 ± 4.1 (**)	84 ± 5.6 (**)	180 ± 11 (**)	99 ± 3.8 (*)	122 ± 6.1 (**)
	50.0	13 ± 5 (**)	31 ± 2.8 (**)	52 ± 3.9 (**)	52 ± 9 (**)	41 ± 2.0 (**)	81 ± 5.4 (**)
Viloxazine	5.0	77 ± 4 (**)	87 ± 3.9 (**)	—	284 ± 13 (**)	117 ± 4.9 (*)	—
	50.0	38 ± 3 (**)	61 ± 3.7 (**)	96 ± 6.0 (**)	135 ± 11 (**)	78 ± 4.0 (**)	126 ± 6.0 (**)

As 100% were considered $(11.2 \pm 1.0) \times 10^{-12}$ M/mg of protein during 5 min 5-HT, $(20.0 \pm 1.7) \times 10^{-12}$ M/mg of protein for 5 min dopamine, $(28.1 \pm 2.3) \times 10^{-9}$ M/mg of protein for 5 min GABA.

(*) Stress compared to control, significant difference.

(**) $p = 0.05$.

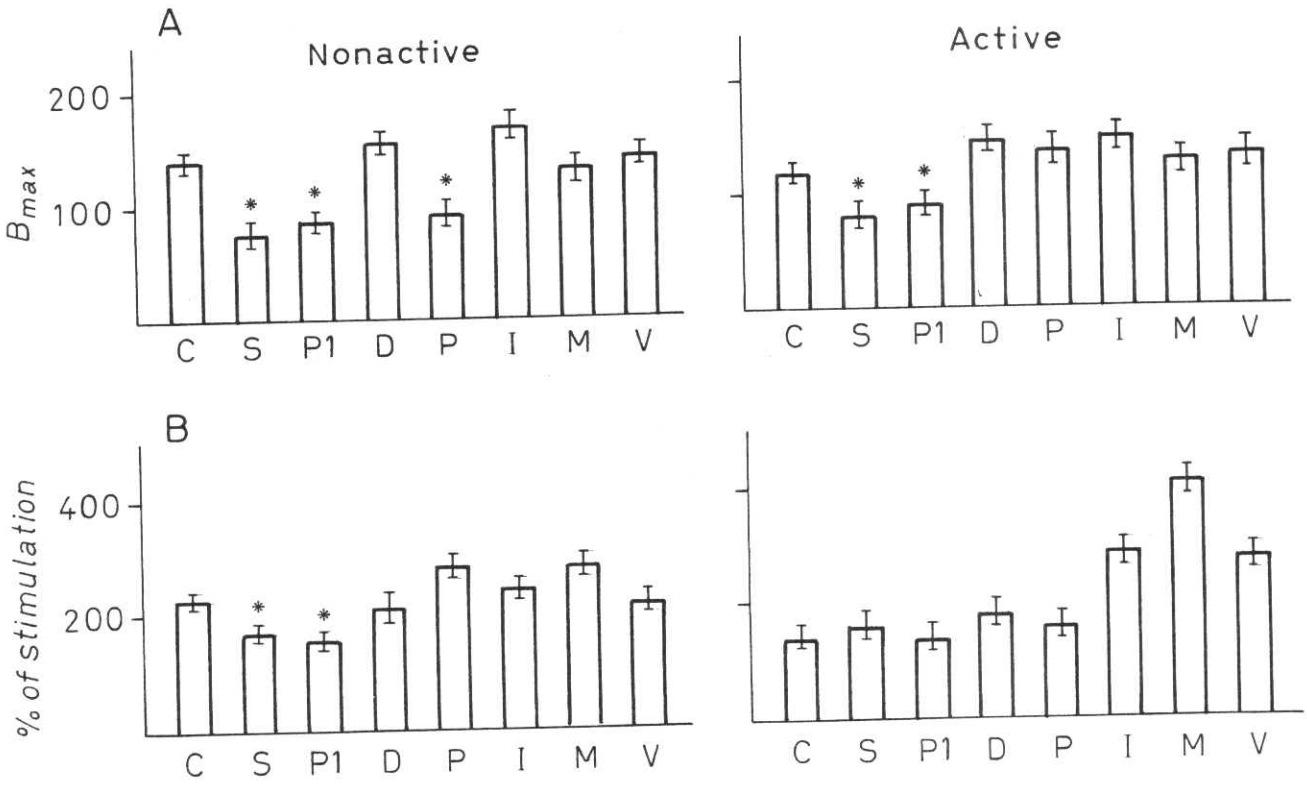

Fig. 1. – Effects of chronic stress and subchronic treatment with psychotropic drugs on beta-adrenoreceptors (A) and coupled adenylate cyclase (B) in active and nonactive rats.

B_{max} – femtamol/mg of protein; cyclase activity – % of stimulation = noradrenaline-stimulated activity/basal activity; activity of cyclase was measured in pmol c-AMP/mg of protein.

C – control; S – stress; P1 – placebo; D – diazepam; P – piracetam; I – imipramine; M – mianserine; V – viloxazine. * – significant differences from control, $p = 0.05$.

Decreased activity of drugs in this test can be due to the increased activity of endogenous antioxidant systems or to the differences in drug's interaction with membranes, because membranes changed their properties. Ability of drugs to inhibit uptake of 5-HT, dopamine and GABA increased proportionally to the increase in activity of each particular uptake system (Table 3).

Characteristics of receptors and coupled adenylate cyclases after chronic stress in active and nonactive rats. – Initially (before stress) there were no differences in the characteristics of studied receptors in both groups of animals (Fig. 1, 2).

After 15 days of chronic psychogenic stress number of all studied receptors and affinity of histamine receptors changed significantly. Differences were found in basal and noradrenaline-stimulated activity of adenylate cyclase in active and nonactive rats. In active animals basal activity of enzyme is higher, but stimulation with noradrenaline is lower than in nonactive ones (Fig. 1). No differences in basal and stimulated activity of histamine-dependent adenylate cyclase were found (Fig. 2).

Subchronic treatment with psychotropic drugs and receptors. – All studied drugs normalized the density of beta-adrenoreceptors in both groups of rats; only piracetam was ineffective in a group of nonactive animals and imipramine produced hyperactivation of receptors in both groups of animals (Fig. 1). Piracetam decreased affinity of beta-adrenoreceptors in nonactive animals. Normalization of histamine receptors (Fig. 2) correlates with the "therapeutic" efficiency of studied drugs. Piracetam and diazepam normalize density and affinity of histamine receptors in active animals, while antidepressants and piracetam are effective in nonactive rats.

Effects of subchronic treatment with psychotropic drugs on adenylate cyclase activity after stress. – Administration of diazepam and piracetam normalizes basal activity of noradrenaline-dependent enzyme in active animals (Fig. 1). Antidepressants significantly increase stimulated activity of adenylate cyclase (by 300-400%), but do not change its basal activity. Diazepam in nonactive rats did not affect neither basal nor stimulated cyclase activity. Piracetam and AD's normalize both parameters to initial level (Fig. 1).

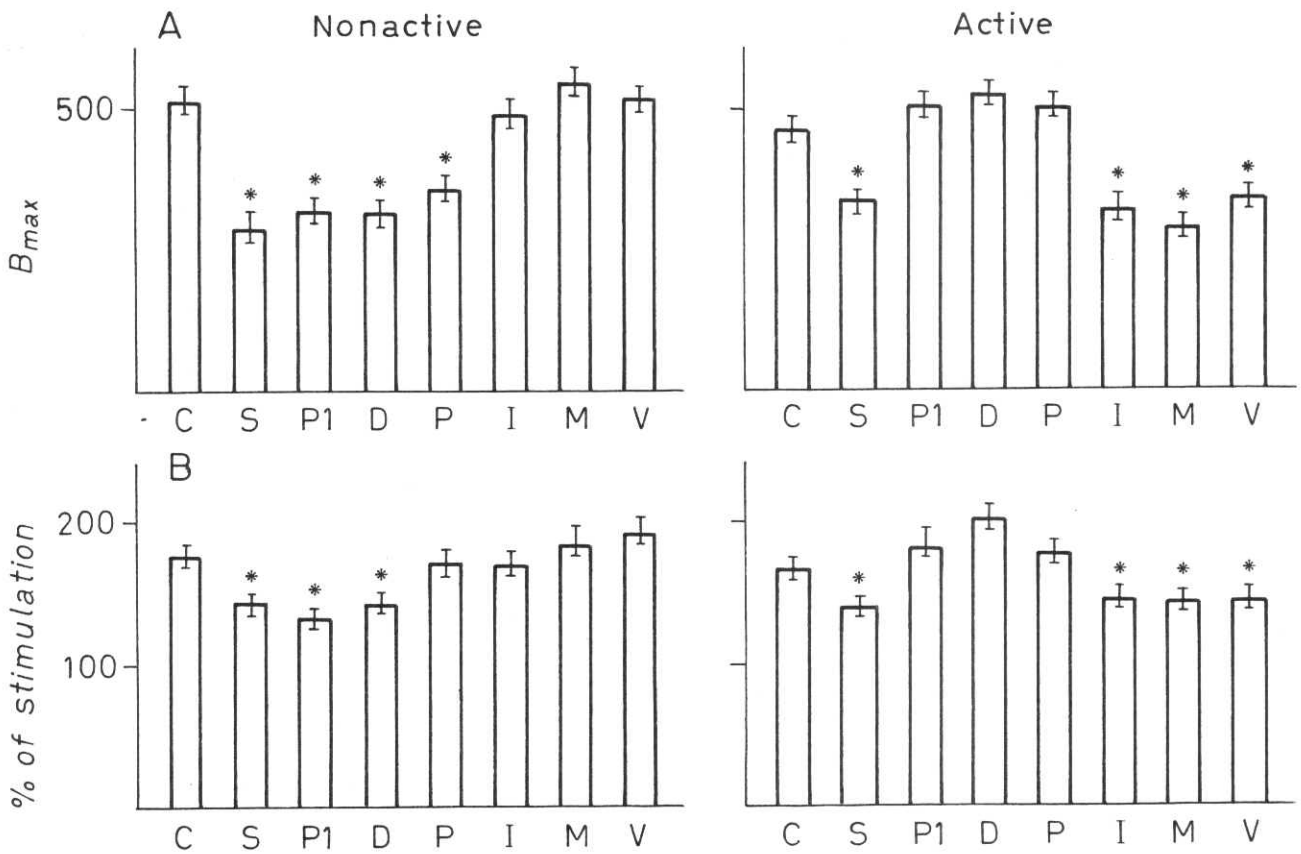

Fig. 2. – Effects of chronic stress and subchronic treatment with psychotropic drugs on histamine receptors (A) and coupled adenylate cyclase (B) in active and nonactive rats.

B_{max} – femtamol/mg of protein; cyclase activity – % of stimulation = histamine-stimulated activity/basal activity; activity of cyclase was measured in pmol c-AMP/mg of protein.

C – control; S – stress; Pl – placebo; D – diazepam; P – piracetam; I – imipramine; M – mianserine; V – viloxazine. * – significant differences from control. $p = 0.05$.

Placebo restores histamine stimulation of adenylate cyclase only in active animals, basal activity in both groups does not change (Fig. 2). Subchronic treatment with diazepam and piracetam normalizes basal and histamine-stimulated activity of the enzyme in active animals. Piracetam, unlike diazepam, is also potent in nonactive rats. AD's restore only histamine-stimulated activity of adenylate cyclase in active animals, in nonactive animals those drugs are potent correctors of enzyme's basal and stimulated activities (Fig. 2).

Discussion

Recently it was reported that chronic psychogenic stress results in profound changes in synaptosomal membranes from rat brain cortex and in activity of membrane-related processes [13].

Analysis of lipid peroxidation processes in synaptosomal membranes from rat brain cortex showed increased level of TBA-active products, but stimulation with prooxidant is lower than in control group. It can be due to increased activity of endogenous antioxidant systems and/or to a decrease in polyunsaturated fatty acid's chains in lipid molecules after chronic stress.

Modification of synaptosomal membranes due to increased lipid peroxidation can cause changes in membrane-bound enzyme's activity (adenylate cyclase, Na, K-ATPase), because increase in membrane's microviscosity decreases activity of Na, K-ATPase [13, 14] , disturbs coupling of receptor and cyclase [15].

Effects of psychotropic drugs on lipid peroxidation and uptake of neurotransmitters after chronic stress are different from those on membranes from control rats. Thus we can conclude that stress alters membranes and that this alteration modifies effects of psychotropic drugs on membrane level *in vitro*.

Subchronic treatment with psychotropic drugs showed peculiarities in correction of stress-induced changes in active and nonactive rats. Piracetam was potent corrector of biochemical parameters in both groups of animals, diazepam — in active animals, antidepressants — in nonactive animals.

Our data indicates significant differences in effects of psychotropic drugs on membranes from control rats and from animals with experimental psychopathology. Summarizing results, we underline that membrane effects of psychotropic drugs should be evaluated on animals with experimental psychopathology and initial reactivity of rats should also be taken into account.

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