Ann. Ist. Super. Sanità
Vol. 26, N. 1 (1990), pp. 31-36

CHANGES IN SYNAPTOSOMAL MEMBRANES FROM CEREBRAL CORTEX DUE TO PSYCHOGENIC STRESS IN RATS

N.A. AVDULOV, A.V. EREMENKO, A.V. VALDMAN, K.M. MALIN, N.R. BULATOVA, A.V. KRINSKAYA, L.D. LUKJANOVA, A.M. DUDCHENKO and A.S. LOSEV

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

Summary. - *The changes in synaptosomal membranes in comparison with those of macrophages and mitochondria during repeated psychogenic stress were evaluated using a specially designed multiprobe procedure. Activity of Na,K-ATPase activity was measured as a functional marker for synaptosomal membranes. The interaction of peritoneal macrophages with nitrobluetetrazole (NBT-test) was also evaluated. Some bioenergetic parameters were measured in mitochondria using spectrophotometric techniques. The data revealed profound structural and functional changes in synaptosomal membranes on 15th day of stress. Those in macrophage membranes, present on 3rd and 6th day were not accompanied by functional ones. In conclusion the present data suggest that stress repeated for 15 days leads to a modelled psychopathology in rats and induce selective structural and functional changes in synaptosomal membranes from the cerebral cortex. This model may be used for investigations of the effects of psychotropic drugs.*

Riassunto (Modificazioni delle membrane sinaptosomiali di corteccia cerebrale in seguito allo stress psicogeno nel ratto). - *Sono state studiate le modificazioni delle membrane sinaptosomiali durante un ripetuto stress psicogeno, utilizzando una procedura multiprobe. In parallelo sono state anche studiate le modificazioni delle membrane dei macrofagi e dei mitocondri. L'attività dell'Na+,K-ATPasi è stata misurata come marker funzionale delle membrane sinaptosomiali. Le modificazioni dei macrofagi peritoneali sono state anche valutate mediante l'interazione con il nitrobluetetrazolo (NBT-test), mentre quelle dei mitocondri mediante la determinazione di alcuni parametri bioenergetici. I dati indicano nelle membrane sinaptosomiali profonde modificazioni strutturali e funzionali al 15° giorno di stress. Le modificazioni strutturali delle membrane dei macrofagi, presenti al 3° e 6° giorno, non erano accompagnate da modificazioni funzionali. In conclusione i dati suggeriscono che lo stress ripetuto per 15 giorni induce nei ratti modificazioni strutturali e funzionali solo nelle membrane sinaptosomiali della corteccia cerebrale. Questo modello può essere usato per ricerche sugli effetti di farmaci psicotropi.*

Introduction

Recent study in this laboratory indicated that repeated psychogenic stress [1] resulted in profound changes in the structure and functions of synaptosomal membranes from rat brain cortex [2]. Stress reaction is a complex one, with different neuro- and biochemical changes, mainly adaptive in their nature [3, 4]. Moreover, there is a close connection between CNS and immune system [5, 6] resulting in involvement of immune response in stress reaction [7]. As concerns biochemical changes, most processes in biological systems require energy and may be also affected by stress. Taking into account all these considerations it appeared of interest to evaluate dynamics of different biomembranes and membrane-related processes during repeated psychogenic stress.

The purpose of the present study was therefore to evaluate the effects of chronic psychogenic stress on the structure and functions of synaptosomal membranes from rat brain cortex. The changes were compared with those in peritoneal macrophages, as immune cells, and with those in brain mitochondria as organelles responsible for bioenergetics.

Materials and methods

Animals, treatment and biological material

All experiments were performed on male albino rats (180-200 g) obtained from the animal house of the USSR Academy of Medical Sciences «Stolbovaya» and kept in the animal house of the Institute of Pharmacology. Repeated stress was in-

duced according to the modified procedure of Mechedova *et al.* [1], as previously described [2].

Rats were decapitated between 1 and 2 pm, 24 hours after the last stress session. Pure fraction of synaptosomal membranes from rat brain cortex was obtained according to the method of Christian [8], as recently reported [2].

Mitochondria of rat brain cortex were prepared according to classical methods [10]. Peritonal macrophages were purified by traditional techniques [9].

Evaluation of changes in biomembranes

Structural changes in biomembranes were analyzed using a specially designed fluorescent multiprobes method, based on inductive resonance energy transfer [8, 11, 12]. Since the transfer is possible only on a certain distance, proportional to Förster radius [11], the efficacy of transfer is strictly proportional and related to distance between fluorophores. Therefore, the calculation of efficacy of transfer between differently located fluorophores allows the evaluation of some structural parameters such as thickness of lipid bilayer, or interlocation of integral proteins [12]. In standard experiments biomembranes (about 50 μg of protein) were added to 1 ml of buffer solution containing 150 mM NaCl, 6 mM KCl, 0.5 mM EDTA, 10 mM Tris-HCl buffer, pH = 7.4. After 2 min of preincubation the spectra of endogenous tryptophan were recorded at λex = 286 nm, λem = 300-350 nm (brand-pass - EX = 3.0, EM = 1.5 nm, EM filter - 290 nm). Then 1 μM of pyrene (Sigma, USA, solution in dimethylformamide) was added slowly (1 μl/min) by microsyringe to each sample. After 5 min the spectra of endogenous tryptophan were recorded again and efficacy of energy transfer from tryptophan to pyrene was calculated by equation: (It - Itp) / It, where It fluorescence of endogenous tryptophan, Itp - fluorescence of tryptophan after the addition of pyrene. Then in the same sample the spectra of pyrene were recorded (λex = 334 nm, λem = 350-500 nm, filter - 350 nm). Pyrene is known to form complexes of excited and nonexcited molecules which are called eximeres (excited dimeres) and their concentration is proportional to the viscosity of the surroundings [11]. Therefore microviscosity was measured as proportional to the ratio of pyrene eximere (Iex, λ = 480 nm) versus pyrene monomer (Im, λ = 373 nm). Subsequently to the same sample 50 μM ANSA (1-anilino-8-naphtalene sulfonic acid; Sigma, USA) was added and after 5 min the intensity of pyrene monomer fluorescence was measured again. The efficacy of energy transfer from pyrene to ANSA was calculated by equation similar to the above mentioned. All fluorescent measurements were performed on Hitachi M-850 (Japan) fluorescent spectrophotometer in thermostated cell holder with continuous stirring and recording in 10 mm quartz cuvettes.

Other biochemical analyses

Na,K-ATPase activity was measured as described by Glebov *et al.* [13]. Adenylate pool was measured both by bioluminiscent method [14] and spectrophotometrically [10]. Adhesion of peritoneal macrophages and nitrobluetetrazole (NBT) test were evaluated as described by other authors [15, 16]. Protein content was measured by the method of Lowry modified by Peterson [17]. Statistical analysis (Student's t-test) and other calculations were performed on «Amstrad PCW 8256» personal computer.

Results

Structural and functional changes in synaptosomal membranes from rat brain cortex

The results of multiprobe analysis of synaptosomal membranes are presented in Table 1. The data indicate a decrease in efficiency of energy transfer from tryptophan to pyrene on day 3. Since the transfer is possible only from the external surface of proteins, the decrease in energy transfer indicates the increased

Table 1. - *Effects of chronic psychogenic stress on the structure of synaptosomal membranes from rat brain cortex*

Days	Treatment	no.	$\frac{It - Itp}{It}$	$\frac{Iex}{Im}$	$\frac{Ip - IpANSA}{Ip}$	F470	F525	F570
3	Control	9	0.7490±0.021	2.18±0.40	0.5799±0.015	25.7±0.76	66.4±0.96	38.0±0.71
	Stress	8	0.7231±0.008(*)	2.09±0.19	0.5634±0.019	25.1±1.39	66.4±1.98	39.2±2.02
6	Control	9	0.7200±0.005	1.93±0.08	0.6003±0.033	25.6±0.55	65.4±1.10	38.2±1.10
	Stress	9	0.6975±0.014(*)	1.67±0.18	0.5728±0.013(*)	25.4±1.37	67.1±1.99	39.0±1.53
10	Control	8	0.6866±0.001	1.74±0.32	0.5556±0.034	24.7±1.77	64.8±3.18	37.5±1.41
	Stress	10	0.7250±0.041	1.93±0.19	0.5703±0.028	25.2±1.12	65.7±1.64	38.0±1.41
15	Control	8	0.7445±0.019	2.24±0.33	0.5748±0.014	25.9±0.65	65.2±0.84	36.6±0.82
	Stress	8	0.7275±0.013	1.89±0.14(*)	0.5858±0.013	25.6±1.34	66.0±1.7	38.4±1.08

no.: number of animals; data expressed as means ± SD; (*): p = 0.05; It: tryptophan fluorescence; Itp: tryptophan fluorescence after the addition of pyrene; Iex & Im: fluorescence of pyrene eximer and monomer; Ip: fluorescence of pyrene monomer; IpANSA: fluorescence of pyrene monomer after the addition of ANSA; F470, F525, F570: fluorescence of dimethylchalcone at 470nm, 525nm and 570nm (Ex = 419 nm).

clusterization of integral membrane proteins. On day 6 the efficacy of energy transfer from pyrene to ANSA decreased. Since ANSA is located on the surface of the membrane, and pyrene in the central part of the hydrophobic core of lipid bilayer, the decrease in efficacy of transfer indicates an increase in membrane thickness. On day 10 membrane structure is not different from control. On day 15 the decrease in pyrene eximerization and spectral shift of dimethylchalcone to longer wavelengths indicates changes in membranes fluidity.

The changes in activity of Na,K-ATPase in terms of Vmax and/or Km are present on days 6, 10 and 15 (Table 2). Differences in control values on various days reflect probably physiological changes of enzymatic activity. From the comparison of structural and functional changes in synaptosomal membranes it appears that on day 3 clusterization of proteins increases without affecting the enzymatic activity. On day 6 there is an increase in the membranes thickness. This probably causes a hindrance of some active sites of the enzymatic molecules resulting in decrease of Vmax. This in turn is compensated by the increase in affinity of enzyme (decrease in Km), and the overall changes do not probably alter the functions. On day 10 the membrane structure is not substantially altered, while elevated Vmax value may be considered a compensatory change consisting of a rise in availability of previously reduced active sites of enzymatic molecules. On day 15 the affinity of enzyme increases, probably in relation to changes in microviscosity of the synaptosomal membranes.

Structural and functional changes in peritoneal macrophages

There are no significant changes in the activity of macrophages in NBT-test while significant changes in percentage adhesion are observed on day 3 and 6 (Fig. 1). Structural changes in macrophage membranes, presented in Table 3, show that the most profound changes in functional activity on day 3 are

Table 2. - *Effects of chronic psychogenic stress on Na,K-ATPase activity in rat brain cortex*

Days	Treatment	no. of animals	Vmax, µmol of Pi/mg protein/h (M ± SD)	Km, × 10^{-4}M (M ± SD)
3	Control	9	46.3± 2.2	5.9±1.2
	Stress	8	39.6± 4.6	5.8±1.5
6	Control	9	90.3±10.4	15.3±3.2
	Stress	9	56.3± 6.1(*)	5.4±2.3(*)
10	Control	8	49.1± 4.8	5.7±0.1
	Stress	10	74.7± 8.9(*)	11.6±3.8
15	Control	8	68.7± 4.9	14.4±2.4
	Stress	8	66.0± 3.3	5.3±1.4(*)

The date expressed as means ± SD; (*) p = 0.05.

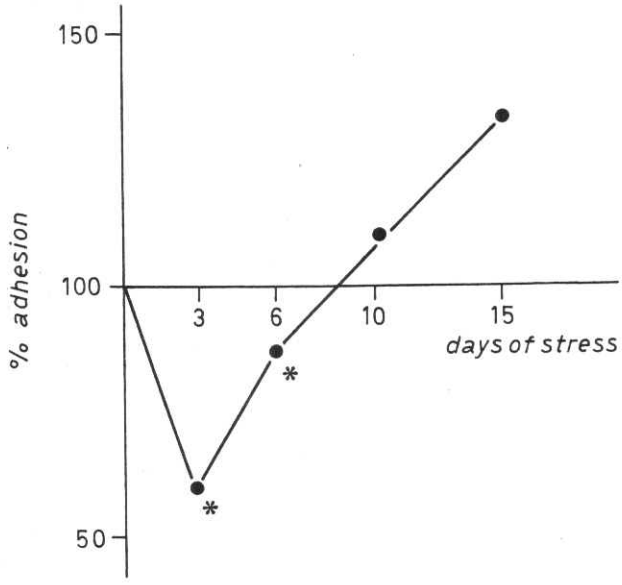

Fig. 1. - Effects of chronic psychogenic stress on percentage adhesion of peritoneal macrophages.

Table 3. - *Effects of chronic psychogenic stress on the structure of macrophages membranes*

Days	Treatment	no.	$\frac{It - Itp}{It}$	$\frac{Iex}{Im}$	$\frac{Ip - IpANSA}{Ip}$	F470	F525	F570
3	Control	9	0.5325±0.012	0.63±0.06	0.5524±0.071	18.2±0.56	60.4±1.03	49.9±0.59
	Stress	8	0.5117±0.015	0.89±0.05(*)	0.5717±0.011	18.9±0.82	61.4±1.82	46.6±1.05(*)
6	Control	9	0.5482±0.007	1.10±0.05	0.5604±0.011	20.0±0.1	66.0±0.1	48.0±0.1
	Stress	9	0.4680±0.022(*)	1.03±0.06	0.5273±0.012(*)	19.5±0.77	65.6±0.56	48.1±0.67
10	Control	8	0.4580±0.009	0.77±0.11	0.4580±0.03			
	Stress	10	0.5004±0.022	1.09±0.11	0.5592±0.02 (*)	21.5±1.54	67.7±1.91	47.3±2.84
15	Control	8	0.5480±0.008	1.20±0.09	0.5754±0.004	18.5±0.1	67.5±0.1	49.5±0.1
	Stress	8	0.4036±0.02 (*)	0.91±0.17	0.2802±0.068(*)			

Note: as in Table 1.

Table 4. - *Some bioenergetic parameters in mitochondria of rat brain cortex following 15-day psychogenic stress*

Adenylate pool

Treatment	no.	ATP	ADP	AMP
		µmol/g of wet tissue		
Control	10	2.2±0.1	0.7±0.04	0.4±0.04
Stress	8	2.2±0.1	0.8±0.06	0.5±0.05

Cytochromes

Treatment	no.	A	B	$C + C_1$
		nmol/g of wet tissue		
Control	9	9.7±0.97	1.89±0.23	7.62±0.73
Stress	10	9.8±0.81	1.87±0.29	9.48±0.01(*)

no.: number of animals; data are expressed as mean ± SD (*) p = 0.05.

accompanied by changes in membrane fluidity; on day 6 the modifications of membranes compensate functional disturbances. The changes, with respect to controls, in membrane structure on days 6 and 15 are rather similar.

Structural and functional changes in brain mitochondrial membranes

The data presented in Table 4 on the mitochondrial bioenergetic parameters indicate significant differences in the levels of cytochromes $C + C_1$ only on day 15. The adenylate pool was not influenced by stress.

Discussion

In psychopharmacological studies most drugs are administered acutely, and almost always to apparently healthy animals. In clinics the same drugs are administered chronically to patients so it appears reasonable to evaluate effects of psychotropic drugs in psychopathological models in animals. On the other hand, all biological processes have their dynamics which consist of adaptation or disadaptation. It is also necessary to take into account that the so called «non-specific» changes in membrane structure, involving physico-chemical properties of membrane lipids can lead to very specific changes in activity of membrane-bound proteins, enzymes and/or receptors. In different tissues the same type of stimulus can lead to different events. In the present study it has been found that chronic psychogenic stress induced structural and functional changes in synaptosomal membranes, but not in macrophages and mitochondria.

The present data show that the increase in membranes viscosity leads to the increase in enzymes activity. Other authors [18] showed that decrease in membranes viscosity leads to decrease in enzyme activities. The overall data show that this type of modulation of enzyme activity (via modulation of membranes fluidity) works both ways. As regards the data on macrophages, it has to be taken into account that the life-time of these cells is of about 5-7 days. Thus the macrophages observed on days 6 and 15 are probably the new ones, with membrane structures modified under stress, resulting in increased thickness and clusterization of proteins. As mentioned above, mitochondrial membranes on day 10 do not differ from controls. Taking into account that the turnover of mitochondria is of about 10-12 days, it can be supposed that on day 10 a new generation of these organelles appear under stress, and more stress-resistant ones are already present. As regards functional activity of these membranes, the stress induced an increased efficiency of the external path of oxidation.

These data speak in favour of some activation in membrane reconstruction processes during chronic stress. As concerns the multiprobe method, it appears a useful and sensitive tool for investigation of structural perturbations in different biomembranes. This allowed to suggest that chronic psychogenic stress leads to structural and functional perturbations in synaptosomal membranes. The same stress induces structural but not functional changes in membranes of mitochondria and macrophages. In conclusion, chronic psychogenic stress leads to modelled psychopathology, not involving immune system or bioenergetics. This model can be utilized for investigations of effects of psychotropic drugs, including dynamics of pathological changes.

REFERENCES

1. MECHEDOVA, A.J., HECHT, K., TREPTOW, K. & HECHT, T. 1976. Zur Rolle der Unbestimmtheit im bedingt-reflektorichen Lernverhalten fur ausgewahlte Herz-Kreislauffunktionen. *Acta Biol. Med. Germ.* **35**: 867-879.

2. VALDMAN, A.V., AVDULOV, N.A., EREMENKO, A.V. & LOSEV, A.S. 1988. Membrane aspects of chronic stress: effects of psychotropic drugs. *Ann. Ist. Super. Sanità* **24**: 437-442.

3. CALABRESE, J.R., KLING, M.A. & GOLD, P.W. 1987. Alterations in immunocompetence during stress, bereavement and depression: focus on neuroendocrine regulation. *Am. J. Psychiatr.* **144**: 1123-1134.

4. BORANIC, M., PERICIC, D., RADIACIC, M., POLIAK-BLAZI, M., SVERKO, V. & MILJENOVIC, G. 1982. Immunological and neuroendocrinic responses of rats to prolonged or repeated stress. *Biomedicine* **36**: 23-28.

5. BESEDOVSKY, M. & DEL REY, A. 1987. Immune-neuroendocrine interactions. In: *International congress on progress in immunology*. B. Cinader & R.G. Miller (Eds). Academic Press, New York. pp. 578-587.

6. BLALOCK, J.E. & WEIGENT, D.A. 1987. Interactions between the neuroendocrine and immune system: common hormones and receptors. A. Moller (Ed.). *Immunol. Rev.* **100**: 79-108.

7. BACH, J.F. & STRONE, T.B. 1985. Corticosteroids. In: The mode of actions of immunosuppressive agents. J.L. Turk (Ed.). *Res. Monogr. Immunol.* **9**: 21-104.

8. CHRISTIAN, S.T. 1973. The interactions of phenothiazine derivatives with synaptosomal membranes: the use of fluorescent probes as an indicator of drug-induced conformational perturbations. *Int. J. Neurosci* **6**: 57-65.

9. NEVEU, P.J. 1986. The mononuclear phagocyte system. *Bull. Inst. Pasteur* **84**: 23-66.

10. MOCHOVA, E.N. & ZHIGACHEVA, I.V. 1977. *Mitochondrias. Accumulation of energy and regulation of enzymatic precesses*. Nauka, Moscow. pp. 139-142 (in Russian).

11. VLADIMIROV, J.A. & DOBRETSOV, G.E. 1980. *Fluorescent probes in biological membranes studies*. Nauka, Moscow. pp. 135-142 (in Russian).

12. AVDULOV, N.A. & EREMENKO, A.V. 1988. Interaction of chlorpromazine and trifluperazine with synaptosomal membranes from rat brain cortex. *Ann. Ist. Super. Sanità* **24**: 477-478.

13. GLEBOV, R.N., SHEVTSOV, V.V., GRISHANKOVA, E.V., NIKUSHKIN, E.V. & KZYJANOVSKYI, G.N. 1971. An investigation of the activity of ATP-ases and monoamineoxidase in subcellular fractions of rat brain in normalcy and electroconvulsions. *Bull. Exp. Biol. Med.* **72**: 36-38 (in Russian).

14. ATAULLAKHANOV, F.I. & PICHUGIN, A.V. 1981. Modification of the luciferin-luciferase method of determining ATP concentration in erythrocytes. *Biophysics* **26**: 86-88.

15. ELFERINK, J.G.R. 1984. Measurement of the metabolic burst in human neutrophils: a comparison between cytochrome C and NBT reduction. *Res. Commun. Chem. Pathol. Pharmacol.* **43**: 339-342.

16. CHESNOKOV, V.A., VOSKRESENSKY, A.M. & SVIRIDOV, L.P., 1985. Evaluation of peritoneal macrophages functional activity with the help of tetrazole test. *J. Microbiol. Epidemiol. Innunobiol.* **5**: 89-90 (in Russian).

17. PETERSON, G.L. 1977. A simplification of the protein assay method of Lowry *et al.* which is more generally applicable. *Anal. Biochem.* **83**: 346-356.

18. BLOJ, B., MORERO, R.D. & FARIAS, R.N. 1973. Membrane fluidity, cholestorol and allosteric transition of membrane-bound Mg^{2+}-ATPase, (Na^+, K^+)-ATPase and acetylcholinesterase from rat erythrocytes. *FEBS Lett.* **38**: 101-105.