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In Vitro Effects of Thyroliberin on Structural State of Plasma Membranes in Mouse Brain and Liver

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The spin probe method was employed to study *in vitro* the effect of regulatory peptide thyroliberin on structural state of surface (0.8 nm) and deep (2 nm) lipid layers of the plasma membranes in mouse liver and brain. Thyroliberin in a concentration range of 10^{-3}-10^{-18} M enhanced structural order of surface lipids, the maximum effect was observed at 10^{-9}-10^{-10} M. The dose-effect dependencies for microviscosity of deep lipids were nonlinear and had 3 extrema at 10^{-4}-10^{-7} M, 10^{-9} M, and 10^{-14}-10^{-16} M. The greatest changes in lipid microviscosity produced by 10^{-9} M thyroliberin are explained by lipid-receptor interaction.

Key Words: *thyroliberin; plasma membranes; microviscosity; structural order*

Thyroliberin (thyreotropin-releasing-hormone, TRH) is an endogenous regulatory peptide involved in the neurohormonal control of some neural and psychic functions (wakefulness, sleep, emotions, learning, and memory) [2]. It alleviates neurological symptoms in patients with brain injuries and contusion [1]. In a broad concentration range including the ultra-low doses (10^{-18}-10^{-4} M) TRH affects spontaneous contractile activity of lymphatic vessels and the level of anticonvulsant protection of the brain during epileptic attacks in humans and animals [1,2]. We previously showed that vascular contractile activity is directly related to changes in the key regulatory element of the LPO system, the structural state (microviscosity) of the surface regions of the lipid bilayer of the endoplasmic reticulum membranes [4,5]. However, during the epileptic seizures, systemic protection is manifested in immediate release of a large amount of TRH in the brain [1,2]. Experimental administration of TRH produces primary changes in the brain, whose plasma membranes (PM) are actively involved in the development of this reaction. In the following, the changes spread over other organs and tissues [12,14].

Our aim was to study *in vitro* the effect of TRH (10^{-18}-10^{-4} M) on the structure of various regions of the lipid bilayer of PM isolated from brain cells (the target for TRH) and liver cells (nonspecific object for TRH).

MATERIALS AND METHODS

The study employed electron paramagnetic resonance (EPR) and the paramagnetic probes based on 5- and 8-doxyl-stearic acid (Sigma). F_1(C57Bl×DBA_2) mice weighing 18-20 g were obtained from Stolbovaya Breeding Department. The TRH solution were prepared by successive 100-fold dilutions of stock solutions (10^{-1} and 10^{-2} M) in distilled water. After decapitation, PM and synaptosomes were isolated according to [10] and [15], respec-

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tively. Protein content was measured by the method of Lowry [11]. The structural parameters of PM were calculated from the spectra obtained on a Bruker-200D EPR-spectrometer by the spin probe method [6]. Stable nitroxyl-labeled radical of 5-doxyl-stearic acid (probe C_5) was used to examine rigidity of the surface regions in lipid bilayer (~0.8 nm), while 16-doxyl-stearic acid (probe C_{16}) was employed to assess microviscosity of the deep layer (~2 nm). Membrane rigidity in the area of localization of C_5 probe was characterized by structural order index S, which reflected mobility of fatty acid chains of lipids in this area. Membrane microviscosity in the area of probe C_{16} localization was assessed by its rotational correlation times τ_{C1} and τ_{C2} calculated by formulas for rapid anisotropic rotation of radicals [6].

The data were processed statistically using parametric and non-parametric tests (Statistica software).

RESULTS

Figure 1 shows the dose-dependent changes of structural order index S in the lipid region located at the depth of 0.8 nm in liver and brain PM. Almost all examined TRH concentrations except 10^{-10}-10^{-11} M significantly increased S in liver and brain PM ($p<0.05$). Index S varies from 0 to 1, and in *in vitro* experiments its changes within 0.3-1.5% are considered as pronounced and important [6]. Qualitatively, the concentration dependencies were similar for PM isolated from both tissues: high concentrations of TRH (10^{-4}-10^{-6} M) significantly increased S, the maximum was attained at 10^{-9}-10^{-10} M and minimum at 10^{-11}-10^{-12} M. In the range of ultra-low concentrations (10^{-12}-10^{-16} M), S increased and attained the maximum at 10^{-16} M. In brain PM (target for TRH), the hormone demonstrated similar membrane-modifying properties at concentrations of 10^{-10} and 10^{-16} M. By contrast, in liver PM the effect of TRH had only one maximum at 10^{-9}-10^{-10} M, its value surpassed that in brain membranes by 2-3 times.

In similar experiments with C_{16} probe on brain and liver PM and on brain synaptosomes, the hormone affected the deep hydrophobic lipid layers. TRH produced similar changes in τ_{C1} and τ_{C2}, which differed only by the absolute values. By this reason, we show only dose-dependencies (% of control) for τ_{C1} (Fig. 2). Significant ($p<0.05$) changes in τ_{C1} were detected within three concentration ranges. TRH in high concentrations of 10^{-4}-10^{-6} M increased τ_{C1} in liver PM, but decreased it in brain PM and synaptosomes (Fig. 2). These changes were maximim at 10^{-9} M. In the concentration range of 10^{-14}-10^{-16} M, changes in τ_{C1} were less pronounced, but significant. The dose-response plots of τ_{C1} for liver and brain PM were virtually symmetrical (Fig. 2, the curves *1* and *2*). It is important that changes in τ_{C1} were unidirectional for brain PM and synaptosomes, and differed only in the amplitude: in brain PM the effect of 10^{-9} M THR 2-fold surpassed that in synaptosomes. Comparative analysis of structural changes in brain and liver PM induced by various concentrations of TRH and assessed with probe C_5 (test for rigidity) and C_{16} (test for microviscosity) in different lipid regions yielded certain inferences. Virtually all examined concentrations of TRH modified the physicochemical parameters of membrane lipids. The corresponding dose-response

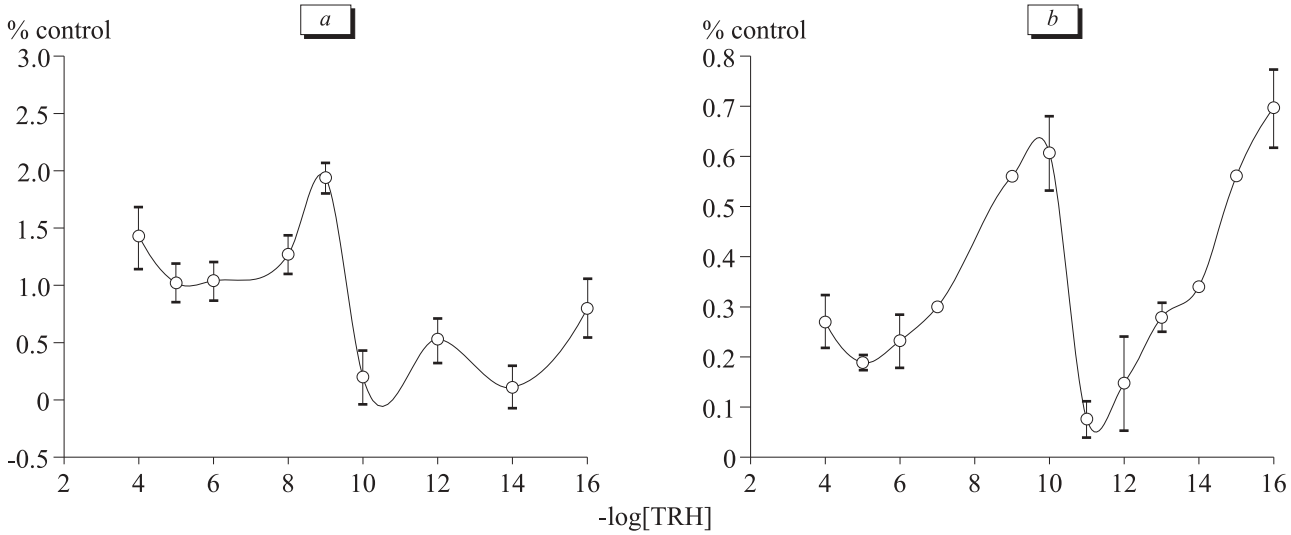

Fig. 1. Effect of TRH on structural order index S assessed with C_5 probe in liver (*a*) and brain (*b*) mouse PM. The concentrations of probe and protein were 10^{-5} M and 4 mg/ml, respectively.

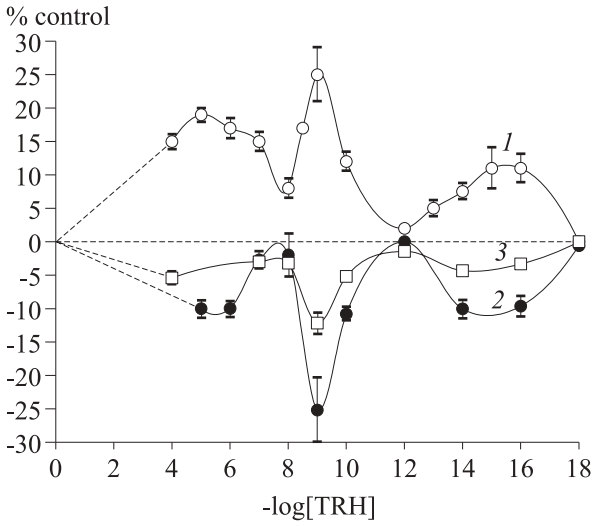

Fig. 2. The dose-response plots of rotational correlation τ_{C1} assessed with probe C_{16} in liver (*1*) or brain (*2*) PM and in brain synaptosomes (*3*). The concentrations of probe and protein were 10^{-5} M and 4.2 mg/ml, respectively.

plots were nonlinear with more (probe C_{16}) or less (probe C_5) expressed three extrema. The effect of TRH on the lipid structure was different in various lipid regions. Near the surface of PM and in the close proximity to phospholipid heads TRH increased rigidity and enhanced structural order both in liver and brain PM (Fig. 1). In the deep lipid layers containing fatty acid chains of phospholipids, TRH induced opposite changes in lipid fluidity in liver and brain PM (Fig. 2). This difference can reflect different fatty acid composition of phospholipids in these PM [13].

The nonlinear character of the dose-response plot is an intrinsic feature of bioactive substances capable to exert their effects at ultra-low concentrations [3]. It can be hypothesized that the effect of high TRH concentrations (10^{-4}-10^{-8} M) results from nonspecific incorporation of this hormone into the plasmalemma. The extremum at 10^{-9}-10^{-10} M (Figs. 1 and 2) is of particular interest. In brain PM and synaptosomes it can be explained by specific interaction with TRH-receptor, which is now isolated, examined, and mathematically modeled [14]. However, in many cases the formation of ligand-receptor complex is accompanied by changes in microviscosity of the lipid regions of PM [7]. According to model [14], TRH initially interacts with amino acids located at the membrane surface, but then the lipid changes spread like a signal into the deep lipid layers. Finally, modification reaches the depth of 20-22 nm, where the nitroxyl fragment of probe C_{16} is situated. This hypothesis explains dramatic changes in τ_{C1} in brain PM under the action of TRH at 10^{-9}-10^{-10} M (Fig. 2, curve *2*). At present, TRH receptor was not isolated from the liver tissues, although expression of its mRNA was demonstrated, which indicated the presence of this receptor in liver. We believe that the character of changes in lipid microviscosity in liver PM (Fig. 2, curve *1*) induced within the concentration range of 10^{-9}-10^{-10} M is an indirect argument in favor of availability of TRH-receptor on liver PM. The effect of TRH at ultra-low concentrations (10^{-12}-10^{-18} M) corresponding to the probabilistic presence of few molecules within a reasonable volume in PM can be explained by the effect of ultra low doses of the chemicals on water structure [3], because TRH is efficiently dissolved in water.

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REFERENCES

1. I. P. Ashmarin, L. M. Asanova, K. R. Abbasova, *et al., Radiats. Biol. Radioecol.,* **43**, No. 3, 324-327 (2003).
2. I. P. Ashmarin, A. P. Kulashev, and S. A. Chepurnov, *Fiziol. Zh*., **75**, No. 5, 627-632 (1989).
3. E. B. Burlakova, A. A. Konradov, and E. L. Mal'tseva, *Khim. Fiz.,* **22**, 21-40 (2003).
4. V. E. Zhernovkov, N. G. Bogdanova, and N. P. Pal'mina, *Biol. Membr.,* **22**, No. 5, 388-395 (2005).
5. V. E. Zhernovkov, T. V. Lelekova, and N. P. Pal'mina, *Radiats. Biol. Radioecol.,* **43**, No. 3, 331-333 (2003).
6. A. N. Kuznetsov, *Spin Probe Method* [in Russian], Moscow (1976).
7. A. S. Polezina, K. A. Anikienko, and V. K. Kurochkin, *Ross. Khim. Zh.,* **43**, No. 5, 72-79 (1999).
8. J. Cao, D. O'Donnell, H. Vu, *et al., J. Biol. Chem*., **273**, No. 48, 32,281-32,287 (1998).
9. M. C. Gershengorn and R. Osman, *Endocrinology*, **142**, No. 1, 2-10 (2001).
10. E. G. Loten and J. C. Redshaw-Loten, *Anal. Biochem.,* **154**, No. 1, 183-185 (1986).
11. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem*., **193**, No. 1, 265-275 (1951).
12. W. Paschen, *Cell Calcium*, **34**, Nos. 4-5, 365-383 (2003).
13. A. A. Spector and M. A. Yorek, *J. Lipid Res.*, **26**, No. 9, 1015-1035 (1985).
14. Y. Sun, X. Lu, and M. C. Gershengorn, *J. Mol. Endocrinol.*, **30**, No. 2, 87-97 (2003).
15. V. P. Whittaker, *Meth. Neurochem.,* N.Y. **2,** 1-52 (1972).