BIOPHYSICS AND BIOCHEMISTRY

Effects of Ultralow Doses of Thyroliberin on Microviscosity of Lipid Components of Biological Membranes

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Effects of the regulatory peptide thyroliberin on microviscosity of lipid components of endoplasmic reticulum biological membranes in mouse hepatocytes were studied by electron paramagnetic resonance. Thyroliberin in a concentration of 10^{-3}-10^{-18} M decreased microviscosity of surface layers of membrane lipids. This decrease was the most pronounced (30%) under effects of 10^{-10} and 10^{-16} M thyroliberin. Physiological effects of thyroliberin corresponded to its influence on the membrane structure.

Key Words: *thyroliberin; biological membrane; microviscosity*

A great body of data shows the effects of biologically active compounds (BAC) in ultralow concentrations (below 10^{-12} M) on living systems [1,2], which are probably associated with their influence on biological membranes and, therefore, various regulatory systems, including cyclic nucleotides, phosphoinositide cycle, and lipid peroxidation (LPO) [7,8,13]. Some BAC, including the modifier of secondary messengers and tumor promoter 12-*o*-tetradecanoylphorbol 13-acetate (TPA) [11,15] and immunomodulator thyroliberin (thyrotropin-releasing hormone, TRH) [9,14], have specific receptors on biological membranes. TPA in ultralow doses markedly changes physicochemical properties of membranes, in particular LPO intensity and microviscosity of lipid components [6]. However, it remains unclear whether or not these physicochemical changes are mediated via ligand-receptor interactions. Taking into account that TRH in physiological concentrations changes microviscosity of lipid components in biological membranes [3], the effects of ultralow doses of TRH on this parameter are of considerable interest.

Here we studied *in vitro* effects of TRH in concentrations of 10^{-18}-10^{-3} M on microviscosity of lipid components of endoplasmic reticulum biological membranes in mouse hepatocytes.

MATERIALS AND METHODS

Thyroliberin was synthesized at the Institute of Organic Synthesis (Latvian Academy of Sciences). Albino SNK mice weighing 18-20 g were obtained from the Stolbovaya nursery. The electron paramagnetic resonance (EPR) probe 2,2,6,6-tetramethyl-4-capryloyloxypiperidine-1-oxyl synthesized at the Institute of Chemical Physics was incorporated into the surface layer of membrane lipids at a depth of 2-4 Å[4]. The mice were decapitated, membranes of mouse hepatocyte endoplasmic reticulum were obtained as described elsewhere [10], and protein content was measured by the method of Lowry [12]. Microviscosity of isolated

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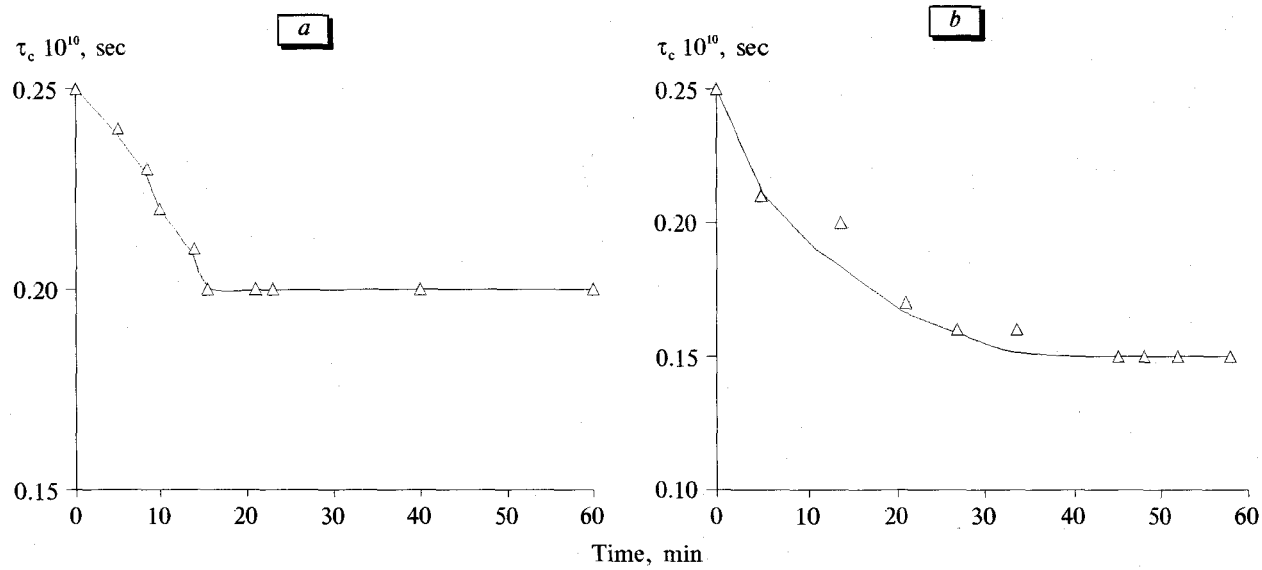

Fig. 1. Typical kinetic curves for rotational correlation time (τ_{sec}) of spin probe in hepatocyte microsomal membranes after administration of thyroliberin in concentrations of 10^{-4} (*a*) and 10^{-16} M (*b*).

membrane fractions was determined by the rotational correlation time (τ_{sec}) of the probe calculated by the formula for spin radicals [4]. Probe concentration was 10^{-5} M. EPR spectra were recorded on a Brucker 2000D spectrometer at 20°C. TRH in various concentrations was added to the membrane suspension after 30-min incubation with EPR probe, and τ_{sec} was recorded at 5-10-min intervals for 1-3 h. The means for 3-7 independent experiments were calculated.

The contractile activity of rat small intestinal mesentery was recorded by intravital biomicroscopy [5]. Experiments with each dose of TRH were performed in 20-30 repetitions.

The results were analyzed using Statistica software.

RESULTS

Figure 1 shows typical kinetic curves for τ_{sec} of the spin probe in microsomal membranes as a function of exposure to TRH. The value of τ_{sec} markedly decreased and reached a plateau 20-35 min after introduction of TRH in high (10^{-4} M, Fig. 1, *a*) and ultralow concentrations (10^{-16} M, Fig. 1, *b*).

Figure 2 shows the dependence of τ_{sec} minimum recorded during the plateau phase on TRH concentration. TRH in all concentrations decreased τ_{sec} ($p<0.05$),

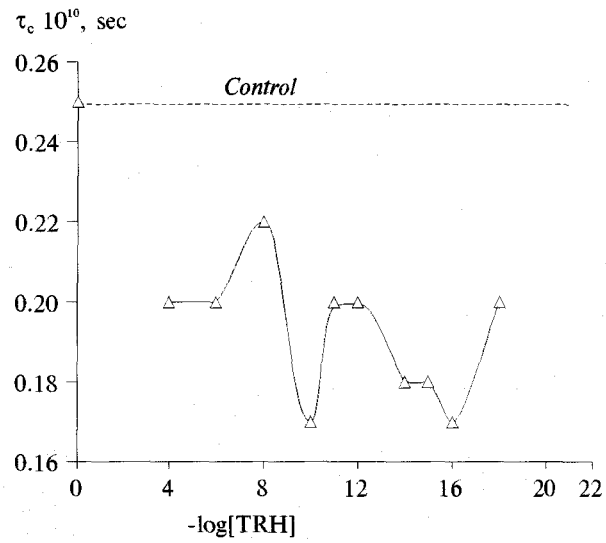

Fig. 2. *In vitro* effects of various concentrations of thyroliberin on rotational correlation time (τ_{sec}) of spin probe in hepatocyte microsomal membranes.

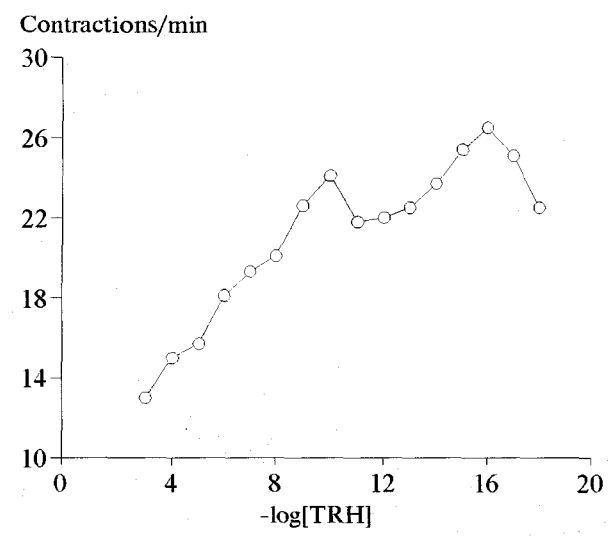

Fig. 3. Contractile activity of lymphatic vessels in rat small intestinal mesentery in the presence of different thyroliberin concentration.

which indicated reduced microviscosity of the surface membrane lipids. It should be emphasized that TRH in concentrations of 10^{-4}, 10^{-6}, 10^{-11}, 10^{-12}, and 10^{-18} M decreased τ_{sec} to the same extent. However, τ_{sec} decreased to a different degree under the effect of 10^{-10} and 10^{-16} M TRH ($p<0.05$).

TRH-induced changes in τ_{sec} were compared with its effects on the contractile activity of lymphatic vessels (Fig. 3). The maximum response ($p<0.05$ compared with the control and adjacent tissues) was observed at TRH concentrations of 10^{-10} and 10^{-16} M. Therefore, physiological effects of TRH corresponded to its influence on membrane structure.

TPA [6] and TRH have specific receptors on cell membranes and modify their structure. TPA decreases fluidity of the surface layers of membrane lipids and, therefore, increases their microviscosity [6], while TRH increases surface fluidity of membrane lipids and, therefore, decreases their microviscosity.

These effects of BAC are probably mediated via ligand-receptor interactions, but the role of other nonspecific factors cannot be excluded.

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