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ANALYSIS OF THE MEMBRANE EFFECTS ON GANGLIOSIDES

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KEY WORDS: gangliosides, biological membranes, fluorescent probes.

One piece of evidence confirming the validity of the concept of neurochemical control over the cerebral circulation [5] was the discovery that gangliosides possess cerebral vasoactivity [6]. It has been shown that an essential role in the effects of gangliosides on the cerebral vessels is played by their interaction with membrane structures [7].

The aim of this investigation was to study the effect of gangliosides on structural lability of the membranes.

EXPERIMENTAL METHOD

Fluorescence spectra were recorded on the MPF-2A spectrofluorometer (Hitachi, Japan) and absorption spectra on the VSU2-P spectrophotometer. If the optical density of the samples was over 0.1, the internal filter effect [9] was taken into account. The quantum yield of fluorescence was measured by a relative method [14] and shifts of the maximum of fluorescence by a two-wave method [10]. The concentration of the free and bound probe was found by calculation [4]. The NO_3^- ion was used as quencher of fluorescence. Accessibility of tryptophanyl residues for quenching was calculated by the method in [2], and the efficiency of energy migration by the method in [1]. Hill's coefficient was determined as the tangent of the angle of slope between Atkinson's coordinates. The total ganglioside fraction was isolated from brain tissue by the method in [12]. Purity of the gangliosides was verified by thin-layer chromatography. Human serum albumin (HSA) from Reanal (Hungary), liposomes obtained from egg lecithin [11], and erythrocyte membranes isolated from fresh donor's blood [13], were used.

EXPERIMENTAL RESULTS

Gangliosides in the initial period of interaction were shown to cause some degree of quenching of fluorescence of 1-anilinonaphthalene-8-sulfonate (ANS), bound with HSA. Later inversion of the effect was observed, to reach a maximum at the 30th minute of contact (Fig. 1a). Quenching of fluorescence of tryptophan residues of HSA by gangliosides depended on the incubation time and the ganglioside concentration (Fig. 1b). A graph of this relationship, plotted between Stern–Volmer coordinates, was concave in form, evidence of cooperativeness

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TABLE 1. Changes in Efficiency of Energy Migration under the Influence of Gangliosides (M ± m)

Donor	Acceptor	Control	Gangliosides	
			$2 \cdot 10^{-4}$ mM	$4 \cdot 10^{-4}$ mM
Tryptophan	ANS	0,31 ± 0,02	0,39 ± 0,02	0,37 ± 0,03
Tyrosine	Tryptophan	0,21 ± 0,02	0,26 ± 0,03	0,26 ± 0,02

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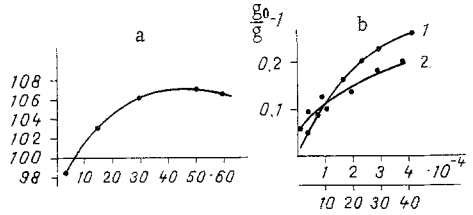

Fig. 1

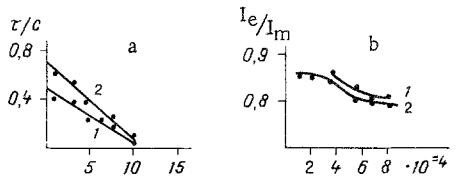

Fig. 2

Fig. 1. Effect of gangliosides (10^{-4} mM) on intensity of fluorescence of ANS bound with HSA (a) depending on incubation time and quenching of fluorescence of HSA tryptophan residues by gangliosides (b), and depending on concentration (1) and duration of contact with protein (2). a) Abscissa, incubation time (in min); ordinate, change in intensity of fluorescence (in % of initial value); b) abscissa, concentration (mM) of gangliosides (top scale) and duration (in min) of incubation (bottom scale); ordinate, ratio of quantum yield of fluorescence of tryptophanyl residues before (g_0) and after (g) action of gangliosides.

Fig. 2. Binding of ANS with liposomes (a) in absence of gangliosides (1) and in their presence in a concentration of $2 \cdot 10^{-4}$ mM (2) and effect of gangliosides on ratio of fluorescence of pyrene excimers and monomers in liposomes (b) before (1) and after (2) preincubation. a) Abscissa, concentration of free probe (τ, in μM) and bound (c) probe; b) abscissa, ganglioside concentration (in mM); ordinate, ratio of intensity of fluorescence of pyrene excimers (I_e) and monomers (I_m).

of interaction between gangliosides and protein. Under the influence of gangliosides conformational changes too took place within the albumin globule, expressed as an increase in migration of energy from tyrosine to tryptophan and from tryptophan to ANS and acridine orange (AO; Table 1), evidence of a reduction of the distance between these elements.

Gangliosides increased the intensity of fluorescence of liposome-bound ANS, with an increase in the ANS–membrane binding constant (Fig. 2a). After incubation of gangliosides with liposomes (at 4°C) for 24 h, their ability to enhance fluorescence of ANS was reduced.

Gangliosides were shown to reduce the rate of diffusion of pyrene in the membrane (Fig. 2b); this effect, moreover, was weaker during short-term contact with liposomes than after 24 h of incubation at 4°C and 1 h of incubation at 37°C.

In the presence of gangliosides the intensity of fluorescence of ANS bound with the erythrocyte membrane increased, with an increase in the probe–membrane binding constant. Intrinsic fluorescence of tryptophan residues also was increased. Meanwhile a short-wave shift of the maximum of fluorescence of ANS and of protein tryptophanyls was observed. This interaction was cooperative in character (Hill's coefficient was 2.8). Gangliosides have the same action on pyrene diffusion in the erythrocyte membrane as in the case of the model membrane.

Gangliosides reduce the accessibility of the protein tryptophan residues for NO_3^-. At the same time the efficiency of migration of energy from tryptophan to ANS and NADH, located on the membrane surface, was reduced, evidence of an increase in the distance between them, possibly due to insertion of the tryptophan residues into the depth of the membrane.

These results suggest that gangliosides affect the conformation of both lipid and protein phases of the membrane, increasing the rigidity of the surface layer, reducing the number of polar and increasing the number of hydrophobic groups, and also reducing the ability of molecules in the microenvironment of the probe and tryptophanyl residues to undergo reorientation during the period of the excited state [3]. A similar effect, as previous investigations showed, is exhibited by gangliosides on microsomal membranes also [8]. By their action on the surface layers of the membrane, gangliosides increase the viscosity of its deeper layers also (data of pyrene diffusion). The existence of this dependence of the action of gangliosides on excimerization of pyrene on the duration of their contact with the membrane indicates that during long contact gangliosides insert themselves into the depth of the membrane.

The experiments thus showed that gangliosides can interact with both proteins and the lipid bilayer. It can be tentatively suggested that gangliosides induce functional structural changes in biomembranes, not by interaction with specific receptors, but as a result of insertion into the lipid phase of the membranes. In turn,

this may also give rise to changes in the conformation of the protein phase, as shown by changes in the parameters of fluorescence of the tryptophanyl residues of the membrane proteins. Direct interaction between gangliosides and membrane proteins also is possible.

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CARDIOTOXICITY OF STROPHANTHIN AND ITS CORRECTION BY ANAPRILIN IN RATS WITH MYOCARDIAL LESIONS OF CORONARY AND NONCORONARY GENESIS AND WITH ACUTE HEART FAILURE

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KEY WORDS: cardiotoxicity of strophanthin, acute heart failure, anaprilin.

A pathological state of the myocardium is one of the important factors which predisposes to glycoside poisoning [1, 7]. The experimental study of changes in sensitivity to the cardiotoxic action of strophanthin in animals with lesions of the myocardium of coronary and noncoronary genesis, during exercise evoking acute heart failure, and investigation of the effectiveness of anaprilin to correct the cardiotoxocity of strophanthin in these conditions, are of great interest.

EXPERIMENTAL METHOD

Experiments were carried out on 177 mature female Wistar rats weighing 160-180 g, anesthetized with thiopental sodium (40mg/kg, intraperitoneally). Sensitivity of the animals to the toxic action of strophanthin K was judged from the minimal arrhythmogenic dose (AD), causing the appearance of the first grouped ventricular extrasystoles or of bigeminy on the ECG, and the lethal dose (LD) leading to cardiac arrest. These doses were established by biological titration, a solution of strophanthin in a concentration of 0.4 mg/ml being injected into the rat's femoral vein from a microburet at the rate of 0.5 ml/min under ECG control in standard lead II. The duration of infusion of the solution in control experiments averaged 12.1 ± 0.4 min.

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