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Interaction of ganglioside-containing planar bilayers with serotonin and inorganic cations

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The binding of serotonin and inorganic cations K^+, Na^+, Ca^{2+}, Mg^{2+} to planar bilayers formed from mixtures of phosphatidylcholine and mono-, di- and trisialogangliosides was studied by the potentiodynamic and nonactin-induced potassium conductivity method. The theoretical analysis of the results obtained was made taking into account (1) protrusion of the ganglioside charges from the membrane surface and (2) simultaneous adsorption of ions on the bilayer surface and on the ganglioside charges protruding into the solution. It was shown that there was no specific binding of K^+ and Na^+. The binding constants for Ca^{2+}, Mg^{2+} were determined. These constants for all the gangliosides studied were equal to 500 M^{-1}. The determined binding constants of serotonin to various gangliosides diminish in the following order: $G_{D3} > G_{T1b} > G_{D1a} > G_{M1}$.

Introduction

One of the possible functions of gangliosides in a cell is the receptors' role. The gangliosides specifically bind bacterial toxins, Sendai virus, lectins, hormones and other physiologically active substances [1,2]. However, for some substances, in particular, serotonin, the receptor function of gangliosides is not sufficiently proven.

Wooley and Gommi [3] had first shown that when a rat stomach muscle preparation was treated with neuraminidase, it lost its sensitivity to serotonin. Subsequent incubation with gangliosides revived the contractibility of the muscle strip, and the most effective ganglioside was G_{D3}. Thus, the authors concluded that it was a receptor to serotonin. But the data from Refs. 4–8 indicate that a receptor to serotonin most probably has a complex nature and can contain gangliosides as coreceptors. This conclusion was confirmed also by investigations on model systems [4,7,9,10]. Despite the contradictory data from different authors, in most cases low binding constants of serotonin to gangliosides were obtained. Moreover, it is shown that there takes place a nonspecific electrostatic interaction of serotonin with the gangliosides and negative charged phospholipids [9].

Affinity of Ca^{2+} to gangliosides is also questionable. It was earlier assumed [11], that the binding of Ca^{2+} to gangliosides could play an essential role in the synaptic transmission of nerve pulses. Data from a number of studies seem to confirm the above assumption: a high value of apparent binding constant of Ca^{2+} to ganglioside micelles was obtained (see, for example, Ref. 12). The affinity of Ca^{2+} to sialic acids was also found to be different, depending on their position in the polar headgroup of a ganglioside [13]. However, in Ref. 14 the interaction of Ca^{2+} with planar bilayers and liposomes formed from mixtures of PC or glycerol monooleate with gangliosides was studied. It was shown that the real binding constant was in the interval from 0 to 100 M^{-1}. In the present work the interaction of K^+, Na^+, Ca^{2+}, Mg^{2+} ions as well as serotonin with planar bilayers formed from mixtures of PC with G_{M1}, G_{D1a}, G_{T1b} and G_{D3} is studied and their binding constants to gangliosides are determined.

Abbreviations: PC, phosphatidylcholine; G_{M1}, galactosyl-*N*-acetylgalactosaminyl(*N*-acetylneuraminyl)galactosylglucosylceramide; G_{D1a}, *N*-acetylneuraminylgalactosyl-*N*-acetylgalactosaminyl(*N*-acetylneuraminyl)galactosylglucosylceramide; G_{T1b}, *N*-acetyneuraminylgalactosyl-*N*-acetylgalactosaminyl(*N*-acetylneuraminyl-*N*-acetylneuraminyl)-galactosylglucosylceramide; G_{D3}, *N*-acetylneuraminyl-*N*-acetylneuraminylgalactosylglucosylceramide; CL, cardiolipin.

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Materials and Methods

Materials. Membranes were formed from egg phosphatidylcholine (PC), cardiolipin (CL) from bovine heart and the gangliosides G_{M1}, G_{D1a}, G_{T1b} from human brain, extracted according to the method described in Ref. 19 and the ganglioside G_{D3} extracted from powdered milk according to Ref. 20. The purity of phospholipids and gangliosides was controlled by the method of thin-layer chromatography. All the lipids studied were chromatographically homogeneous substances. According to the thin-layer chromatograms, serotonin in the form of creatinine sulphate and serotonin hydrochloride (Calbiochem, U.S.A.) contained negligible impurities. A methanolic solution of nonactin (Serva, F.R.G.) was used. Inorganic salt solutions were made from recrystallized KCl, NaCl, $CaCl_2$ and $MgCl_2$ solved in distilled or bidistilled water.

The pH values of electrolyte and serotonin solutions (1 mM serotonin in 1 mM KCl solution) were kept near 6.8 ± 0.2 by adding 1 M KOH solution. A mixture of 1 mM KCl and 10^{-6} M ethylenediaminetetraacetate (EDTA) solution was used as background electrolyte.

Distilled chloroform, methanol and decane were used as lipid solvents. Vacuum-dried mixture of PC with gangliosides first was solved in chloroform then decane was added and the mixture was heated up to 40–45 °C for a few minutes until chloroform was removed. In some cases lipids were solved in a mixture of chloroform with methanol and decane (2:1:2, v/v). The concentration of lipids in the membrane-forming solution was 15–20 mg/ml (the solutions were prepared just before the experiment). Bilayers were formed by dropping the lipid solution on a hole of diameter 1 mm in the partition of a two-chamber teflon cell of volume 20 ml. In the experiments with serotonin we used a smaller cell (3 ml). The cell was thermostatically controlled at 30 °C, the solutions were continuously mixed by a magnetic agitator.

Measurement of the bilayer surface potential change. Ag|AgCl electrodes connected via agar-agar bridges with the solutions on both sides of the membrane were used for the measurements. The change of the surface potential of bilayers was determined by two methods. The difference of surface potentials, $\Delta\varphi$, on both sides of the membrane with one-side adding of concentrated solutions of corresponding salts was measured by the potentiodynamic method [21]. The change in the surface potential of bilayer after symmetrical adding of Na^+, Ca^{2+}, Mg^{2+} ions was determined by the method of nonactin-induced potassium conductivity [22]. The nonactin concentration in both chambers of the cell was $1.25 \cdot 10^{-5}$ M The conductivity was measured by applying 20 mV to the membrane. As the data of these two methods were the same in all the experiments, hereafter only the experimental dependences obtained by the potentiodynamic method will be presented. To measure the surface potential of ganglioside-containing bilayers in a cell of special construction we caused a PC bilayer to contact with a membrane from a PC/ganglioside mixture and realized their monolayer fusion. The construction of such a cell, the experimental conditions and the technique of measurements were described in Refs. 26 and 27.

Results

The characteristic size of ganglioside polar headgroups is about 2 nm, and the sialic acid charges protrude from the bilayer's surface into the solution by about 1 nm [14,15] (see Fig. 1b). Owing to this fact, the potential distribution at the surface of ganglioside-containing membranes has a complex profile shown in Fig. 1a (see also Ref. 16). There is no analytical solution of the Poisson-Boltzmann equation for the case of protruding charges [14,16,17]. However, in the region of low potentials this equation has an analytical solution [14,16], and the potential at the O($\varphi(o)$) and $a(\varphi(a))$ planes (Fig. 1a) can be obtained from the equations analogous to those in Ref. [14]:

$$\varphi(o) = \frac{\sigma_o}{\varepsilon_o \varepsilon_s \kappa} + \frac{\sigma_a \cdot e^{-\kappa a}}{\varepsilon_o \varepsilon_s \kappa} \tag{1}$$

$$\varphi(a) = \frac{\sigma_o \cdot e^{-\kappa a}}{\varepsilon_o \varepsilon_s \kappa} + \frac{\sigma_a (1 + e^{-2\kappa a})}{2\varepsilon_o \varepsilon_s \kappa} \tag{2}$$

where σ_o and σ_a are the charge densities at the surface of a bilayer and on the plane of a protruding charge, respectively; ε_s and ε_o are the dielectric permeability of the solution and vacuum, respectively; κ^{-1} is the Debye length in the solution; a is the distance between the bilayer's surface and the plane of the protruding charge.

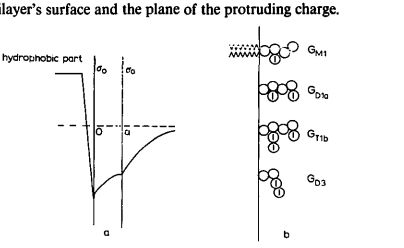

Fig. 1. (a) Potential distribution on the membrane/solution interface for bilayers formed from mixtures of phospholipids with gangliosides. O is the plane of localization of phospholipid polar headgroups, a is the ganglioside charges localization plane, σ_o and σ_a are the densities of surface charges on these planes. (b) Schematic representation of ganglioside molecules (the circles with '−' show the sialic acid residues).

In case of adsorption of Ca^{2+} or Mg^{2+}, the expressions for σ_o and σ_a, which include their binding constants, have the form given in Ref. 14. We have used the approach offered in Ref. 16 to describe the experimental dependences obtained in a wide range of potentials $\varphi(o)$ and $\varphi(a)$. To plot the solid curves shown in Figs. 2–4, we have used the approximate formula which takes the charge protrusion into account:

$$\Delta\varphi = \varphi_b - \varphi(a)\cdot\exp(-\kappa a) \qquad (3)$$

where φ_b is the surface potential of a bilayer in 1 mM KCl solution. $\varphi(a)$ is determined by the Gouy-Chapman equation [18]. The experimental dependences of $\Delta\varphi$ on the Ca^{2+} and Mg^{2+} concentration for bilayers formed from PC/G_{M1} mixtures (5 mol%) as well as the theoretical curves of adsorption of these cations are presented in Fig. 2. The solid theoretical curve is plotted according to the Eqn. 3 regardless of the specific adsorption on PC at the binding constant of Ca^{2+} to G_{M1} ($K_{G_{M1}}$) equal to 500 M^{-1}. However, it is known that calcium specifically binds to PC [23,24,25] (see also Fig. 2). Thus, we can exactly describe the adsorption of Ca^{2+} within 10^{-6}–10^{-4} M, where binding with PC can be neglected. Nevertheless, the solid curve suitably describes the experimental points in the whole range of calcium concentrations. The dashed curve in Fig. 2 is obtained from Eqns. 1 and 2 with regard to adsorption of Ca^{2+} on G_{M1} and PC with binding constants $K_{G_{M1}}$ = 500 M^{-1} and K_{PC} = 200 M^{-1}, respectively. As is seen from Fig. 2, this curve describes the experimental

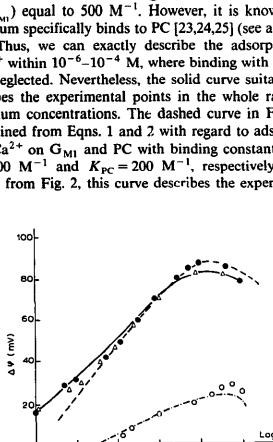

Fig. 2. Dependence of the surface potential difference on concentration of Ca^{2+} (●) and Mg^{2+} (△) for bilayers formed from PC/G_{M1} mixture. The standard errors of $\Delta\varphi$ do not exceed the size of the symbols throughout in the figures. The background solution concentration is 1 mM KCl, pH = 6.8±0.2. The open circles show analogous dependence for bilayers formed from PC. The solid theoretical curve is plotted at a = 1.5 nm, $\sigma_a = -0.011$ K/m^2 ($\varphi_b = -77$ mV), $K_{G_{M1}} = 500$ M^{-1}. The dashed curve is plotted in assumption that $\sigma_a = -0.009$ K/m^2 ($\varphi_b = -70$ mV), $K_{G_{M1}} = 500$ M^{-1}, K_{PC} = 200 M^{-1}. The dash-dotted curve is plotted at the same binding constant to PC (K_{PC} = 200 M^{-1}), as was previously shown in Ref. 31.

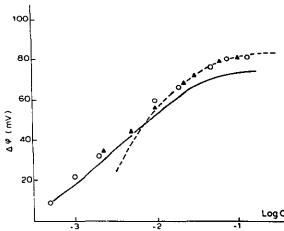

Fig. 3. Dependence of the surface potential difference on concentration of K^+ (▲) and Na^+ (○) for bilayers formed from the PC/G_{M1} mixture. The solid curve is plotted at the same parameters as the solid curve in Fig. 2 is. The dashed curve is plotted in assumption that $\sigma_a = -0.009$ K/m^2 ($\varphi_b = -70$ mV), a = 1.5 nm, $K_{PC} = 8$ M^{-1}.

dependences well enough in the concentration range from 10^{-5} to 10^{-1} M Ca^{2+} despite the fact, that in this interval the value of $\varphi(a)$ is not always low enough. Note, that at the same value of K_{PC} one can satisfactorily describe the dependence $\Delta\varphi$ (log Ca^{2+}) for bilayers formed from PC (dash-dotted curve in Fig. 2). The theoretical description of the experimental dependences shown in Fig. 2 yields the same value of $K_{G_{M1}} = 500$ M^{-1} both in the range of low potentials (high calcium concentrations) taking into account of adsorption on PC and G_{M1}, and in the concentration range from 10^{-6} to 10^{-4} M.

In Fig. 2 the experimental points for Ca^{2+} and Mg^{2+} are practically the same, and this fact gives identical binding constants for these two cations and indicates the absence of Ca^{2+}/Mg^{2+}-specificity to G_{M1}. This is also characteristic of G_{D1a} and G_{T1b} (no data presented).

Fig. 3 shows the experimental dependence of $\Delta\varphi$ on the K^+ and Na^+ concentrations for bilayers formed from the PC/G_{M1} mixture (5 mol%). Note the coincidence of these dependences. The solid theoretical curve is plotted according to Eqn. 3 at the same values of σ_a and a, as in case of the solid curve in Fig. 2 at zero binding constant of K^+ (or Na^+) to G_{M1}. A better agreement to the experiment at high concentrations of the cations studied was obtained from the Eqns. 1 and 2 (dashed curve), assuming that K^+ binds specifically to the bilayer surface with a constant of 8 M^{-1}. Thus, one can consider that monovalent cations do not specifically bind to G_{M1}.

Fig. 4 shows the dependences $\Delta\varphi$(log K^+) and $\Delta\varphi$(log Ca^{2+}) for bilayers formed from the mixtures of PC with G_{D1a} (4.6 mol%) and G_{T1b} (3.9 mol%). The experimental points for K^+ and Na^+ as well as for Ca^{2+} and Mg^{2+}, like in case of a PC/G_{M1} membrane, coincide

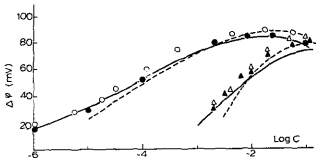

Fig. 4. $\Delta\varphi$ as a function of concentration of mono- and bivalent ions for bilayers formed from PC with G_{D1a} (○, △) and G_{T1b} (●, ▲); △, ▲ $-K^+$; ○, ● $-Ca^{2+}$. The theoretical curves are obtained at the same values of parameters as those in Figs. 2, 3.

(no data presented). The comparison of the curves from Figs. 2–4 shows that dependences of $\Delta\varphi$ on the concentration of bivalent and monovalent cations are similar for all the ganglio sides. That is why the charge densities obtained during the experimental data processing, practically coincide for G_{M1}, G_{D1a} and G_{T1b}, regardless the different charge densities of these gangliosides obtained from their content in the membrane-forming mixtures. Consequently, the values of the

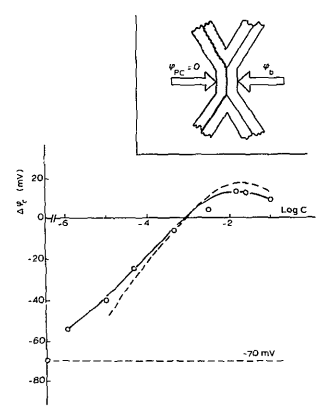

Fig. 5. Measurement of the surface potential difference of the contact bilayer and dependence of $\Delta\varphi_c$ on concentration of Ca^{2+}. The point on the ordinate axis corresponds to φ_b (1 mM KCl solution). $CaCl_2$ is added from the side of the charged monolayer formed from the PC/G_{M1} mixture. The theoretical curves are plotted at the same parameters as those in Fig. 2. Inset. Schematic representation of the contact bilayer obtained as a result of monolayer fusion of the neutral (shaded area) and the charged membranes.

binding constants of G_{D1a} and G_{T1b} to $Ca^{2+}(Mg^{2+})$ are equal to 500 M^{-1}.

For bilayers formed from the mixture of PC with G_{D3}, the processing of the experimental dependences of $\Delta\varphi$ on the K^+ and Ca^{2+} concentrations (no data presented) yields the same value of 500 M^{-1}.

The values of σ_a and a we have so far determined by fitting the theoretical curve to the experimental points of Figs. 2–4. To directly measure the surface potentials of bilayers formed from PC/G_{M1} in 1 mM KCl solution, we carried out a series of special experiments. A bilayer formed from PC was brought to a contact with a bilayer formed from PC/G_{M1} (5 mol%), as described earlier [26,27]. As a result of monolayer fusion of the two membranes, an asymmetric contact bilayer was formed, one of the monolayers of which consisted of PC and the other one consisting of the mixture of PC with G_{M1} (Fig. 5, inset). The surface potential difference on both sides of the contact bilayer, $\Delta\varphi_c$, must be equal to the surface potential of the membrane formed from PC/G_{M1} (accurate to the difference in the dipole potential jumps in PC and mixed monolayers [28]). The value of $\Delta\varphi_c$ measured by the technique described above is equal to -70 ± 10 mV (Fig. 5), which agrees to the value of $\varphi_b(\sigma_a = -0.009$ K/m^2) with the help of which we have already described the experimental dependences shown in Figs. 2–5. Note, that the dependence of $\Delta\varphi_c$ on the Ca^{2+} concentration (Fig. 5) is similar to the analogous dependence shown in Fig. 2 for a usual membrane from PC/G_{M1} mixture. It is seen from Fig. 5 that at calcium concentrations higher than 1 mM the sign of $\Delta\varphi_c$ changes to positive, i.e. the PC/G_{M1} monolayer surface potential becomes higher than zero. The theoretical curves plotted at the same as in Fig. 2 values of K_{GM1}, σ_a and a describe the experimental data satisfactorily.

Binding of serotonin to gangliosides

Serotonin as a salt with creatininesulphate or in the form of hydrochloride added to membranes formed from mixtures of PC with gangliosides yields coinciding dependences of $\Delta\varphi$ on serotonin concentration (no data presented). Hence, one can conclude that creatinine does not specifically interact with gangliosides. Check experiments have shown that serotonin is specifically sorbed on PC (Fig. 6a). The theoretical analysis of the dependence shown in Fig. 6a (▼, curve 1) yields a binding constant of 10 M^{-1} obtained in terms of the Gouy-Stern model. To simplify the theoretical description of serotonin adsorption on ganglioside-containing bilayers, further we shall neglect its interaction with PC because of the low binding constant.

As is seen from Fig. 6a, the dependence of $\Delta\varphi$ on serotonin concentration for the bilayers containing G_{M1} and G_{D1a} are similar. The theoretical analysis of experimental data yields the values of $K_{GM1} = 150$ M^{-1} and

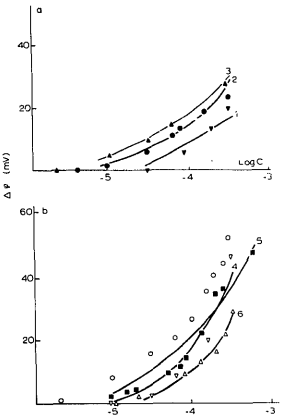

Fig. 6. Experimental and theoretical dependences of the surface potential difference on the serotonin concentration: (a) for bilayers formed from PC (▼, curve 1) and from mixtures of PC: with G_{M1} (●, curve 2) and with G_{D1a} (▲, curve 3); (b) for bilayers formed from mixtures of PC: with G_{T1b} (▽, curve 4), G_{D3} (■, curve 5), CL (△, curve 6) and with total fraction of brain gangliosides (○). The surface charge density for all the ganglioside is assumed to be -0.009 K/m^2, except for PC/G_{D3} ($\sigma_s = -0.006$ K/m^2) and for PC/CL $\sigma_s = -0.028$ K/m^2. Comments are in the text.

$K_{G_{D1a}} = 200$ M^{-1}, the stoichiometry of binding of serotonin to sialic acid being 1 : 1 (curves 2 and 3). The curves 4 and 5 fit to the experimental data best, when the stoichiometry of binding of serotonin to G_{T1b} is 3 : 1 ($K_{G_{T1b}} = 10^8$ M^{-3}) and to G_{D3} is 2 : 1 ($K_{G_{D3}} = 5 \cdot 10^6$ M^{-2}) assuming simultaneous binding of three of two molecules of serotonin, respectively. The calculation of an analogous dependence for a membrane formed from PC/CL (5 mol%, curve 6) yields $K_{CL} = 500$ M^{-2} with stoichiometry 2 : 1. Fig. 6b also presents the experimental points for bilayers formed from mixtures of PC with the total fraction of brain gangliosides.

Discussion

The results of the experiments considered prove the absence of specific binding of monovalent cations with gangliosides (Figs. 3 and 4). This fact agrees to the data of other authors [15,16,29]. The obtained binding constants of all the studied gangliosides to bivalent ions (500 M^{-1}) are close to those calculated for Ca^{2+} in Ref. 14. The low binding constants and the absence of Ca^{2+}/Mg^{2+}-specificity of binding contradict to the assumption that the gangliosides act as receptors for Ca^{2+} [11]. Resemblance of the dependences of $\Delta\varphi$ on K^+ and Ca^{2+} concentrations (Figs. 2–4) indicates the similar binding of Ca^{2+} to sialic acid residues, regardless their position in the polar headgroup of gangliosides (see also Ref. 14). In contrast to the data of Ref. 30, our data suggest similar charge densities for mono-, di- and trisialogangliosides in a bilayer, while, their content in the membrane-forming mixture is 5.0, 4.6 and 3.9 mol%, respectively. Note, that the calculated charge density of bilayers formed from PC/ganglioside mixtures solved in decane and chloroform/methanol/decane mixture (2 : 1 : 2, v/v) were the same and were equal to -0.009 K/m^2. Thus, the phenomenon described above cannot be explained by poor solubility of gangliosides in decane. In Ref. 29 the values of surface potentials for planar bilayers formed from mixtures of PC with G_{M1}, G_{T1b} are also lower than the theoretical ones calculated in accordance with the molar portions of these gangliosides in the membrane-forming solution.

In this paper we offer a method of direct measurement of the surface potential of planar bilayers by measuring the surface potential difference on an asymmetric contact bilayer formed by monolayer fusion of a neutral and a charged membrane. Coincidence of $\Delta\varphi_c$ in 1 mM KCl solution with the value of $\varphi_b = -70$ mV obtained in the theoretical description of the experimental points in Figs. 2–5, allows one to believe that the method proposed gives correct values of surface potentials. Note, that the value of $\varphi_b = -70$ mV obtained for planar bilayers formed from PC/G_{M1} 5 mol% mixture is close to the value of the ζ-potential of liposomes of the same lipid composition in 1 mM NaCl solution, measured in Ref. 14.

The affinity of serotonin to gangliosides studied in this work was essentially different. To compare the obtained constants, it is necessary to bring them to the same units (M^{-1}). We have calculated the concentration of serotonin, when it binds to half of the G_{D3} and G_{T1b} molecules at a given binding constant. The inverse value of this concentration can, we believe, characterize the affinity of G_{T1b} and G_{D3} to serotonin and will enable us to compare their constants with those of other gangliosides. Such estimation yields $K = 4000$ M^{-1} for G_{T1b} and $K = 7800$ M^{-1} for G_{D3}. In case of cardiolipin the binding constant is as low as 100 M^{-1}. It should be noted that the values of the binding constants may be slightly overestimated, because we have neglected the binding of serotonin to PC.

According to our estimates G_{D3} has the highest affinity to serotonin, this agreeing with the data of Wooley and Gommi [3]. As to the receptor function of gangliosides, in particular G_{D3}, the binding constants obtained in this work testify against this assumption, for their values are not sufficiently large.

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