EVIDENCE FOR A SPECIFIC INTERACTION BETWEEN GT_1 GANGLIOSIDE INCORPORATED INTO BILAYER MEMBRANES AND THYROTROPIN

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Received 14 November 1977

1. Introduction

In the past few years, it has become evident that recognition properties of natural cell membranes can be duplicated in model membranes [1–9]. This approach allowed us to demonstrate that glycolipids incorporated in planar lipid bilayers can act as concanavalin membrane receptor [10]. Recent studies suggest that a ganglioside or a ganglioside-like structure may be an important component of the thyrotropin (TSH) membrane receptor [11,12]. The interaction of TSH with the specific ganglioside would result in a conformational modification such that a subunit of the hormone molecule would penetrate the membrane and initiate the adenylate cyclase stimulation [11,12].

The present report demonstrates a specific interaction between TSH and GT_1 ganglioside incorporated in a planar bilayer membrane. The membrane conductance changes support the hypothesis that after specific binding to GT_1 ganglioside, the TSH molecule undergoes a conformational change which leads to a penetration of the hormone in the lipid layer.

2. Materials and methods

Thyrotropic hormone (TSH), glycerol monooleate (GMO), *N*-acetyl galactosamine and *N*-acetylneuraminic acid were Sigma Chemical Co. products. GT_1 ganglioside (*N*-acetylneuraminylgalactosyl-*N*-acetylgalactosaminyl-(*N*-acetylneuraminyl-*N*-acetylneuraminyl)-galactosylglucosylceramide), GD_{1a} ganglioside (*N*-acetylneuraminylgalactosyl-*N*-acetylgalactosaminyl-(*N*-acetylneuraminyl)-galactosylglucosylceramide) and GM_1 ganglioside (galactosyl-*N*-acetylgalactosaminyl-(*N*-acetylneuraminyl)-galactosylglucosylceramide) were Supelco products. Lipids were checked for purity by thin-layer chromatography.

Lactose, glucose and galactose were 'pro analysi' products from Union Chimique Belge (Bruxelles, Belgique). *N*-Decane, a reagent grade product was redistilled before used [13]. The mixtures GMO–gangliosides were dissolved in a chloroform/methanol/decane (30/5/65) mixture. Bilayers were formed on a 1.3 mm diam. aperture in a Teflon cell separating two aqueous phases (2.5 cm^3 each). The aqueous phase contained 0.15 M NaCl + 0.05 M Tris–HCl at pH 7.3; the temperature was maintained at 27°C. The membrane conductance G_m was determined by measuring the specific current I_m/cm^2 as a function of imposed potentials differences V_m, with a 602 Keithley electrometer. The complete system was enclosed in a Faraday cage. The membrane formation was observed under reflected light with a low power microscope.

3. Results and discussion

Conductance changes of GMO planar membranes containing GT_1, GM_1 or GD_{1a} were measured after addition of TSH in the aqueous phase (table 1). A 16-fold increase of conductance was observed in presence of GT_1 in the membrane. No significant effect was obtained with GM_1 and GD_{1a} gangliosides. The sequence of interaction is identical to the sequence of TSH-binding inhibition to thyroid membranes.

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Table 1
Effect of TSH on the conductance of planar membranes containing gangliosides

Bilayers	Conductance (10^{-7} Ω^{-1} cm^{-2}	
	Without TSH	With TSH[a]
GMO	0.20 (9)[b]	0.22 (6)
GMO–GT_1 Molar ratio 97/3	2.4 (16)	40 (11)
GMO–GM_1 Molar ratio 97/3	0.7 (6)	1.2 (10)
GMO–GD_{1a} Molar ratio 97/3	1.1 (6)	1.3 (6)

[a] TSH concentration 120 μg/ml
[b] No. of experiments in brackets

Indeed, GT_1 inhibits strongly the TSH binding, but the inhibition is nearly eliminated with GD_{1a} and GM_1 [11]. Fluorescence studies indicated that TSH–GT_1 interaction induced a conformational change which should lead to the stimulation of the adenylate cyclase by thyrotropin via penetration of the hormone in the membrane [11].

Our results strongly support this hypothesis. Indeed, the magnitude of the effect on the GMO–GT_1 bilayer conductance indicates a modification inside the hydrophobic part of the bilayer. This perturbation is attributed to a partial penetration of the hormone in the membrane. Because no change was observed with GMO bilayers (table 1), a penetration due to the hydrophobicity of the TSH proteins can be ruled out. One can thus suppose that TSH–GT_1 interaction induces an hormone conformational change allowing the penetration of thyrotropin in the bilayer.

If the TSH–GT_1 interaction is specific, it should be possible to reverse it using saccharides present in the hydrophilic moiety of the ganglioside. So, to rule out the possibility of non-specific binding, experiments were carried out in the presence of an equimolar mixture of the saccharide residues and sialic acid groups present in the hydrophilic moiety. Figure 1 shows that in these conditions the TSH effect is completely suppressed.

In order to determine the contribution of each saccharide in the inhibition process, they were tested independently. As shown in table 2, the recognition does not seem to be associated as for lectin molecules with a well defined saccharide group but requires a more complex saccharide structure. Even if a more important inhibition was obtained with glucose, *N*-acetylgalactosamine and *N*-acetylneuraminic acid it would be premature to draw conclusions about the structure of the receptor site.

The fact that saccharides can partially or completely reverse the GT_1–TSH interaction supports the conclusion that thyrotropin interacts specifically with the carbohydrate moiety of the ganglioside. These results confirm qualitatively a recent report describing the preparation of brain gangliosides (12% GT_1) containing liposomes which bind TSH [14]. Our data

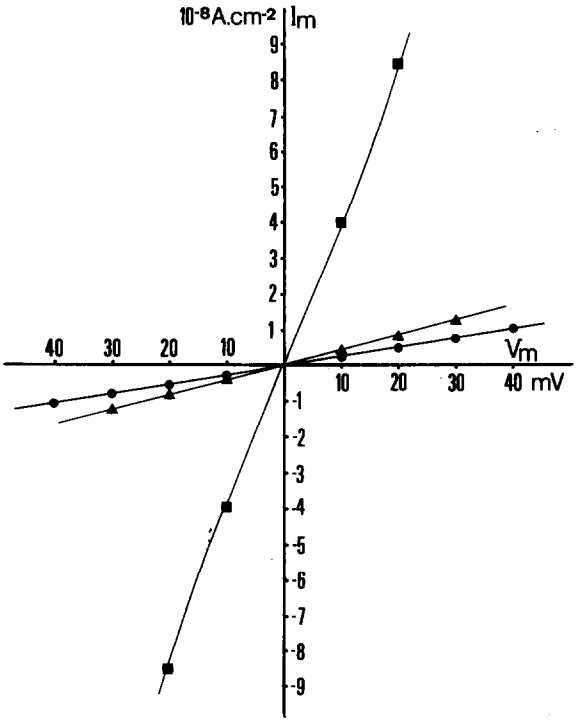

Fig.1. Current–voltage characteristics of GMO bilayers containing GT_1 ganglioside in the absence of TSH (•), in the presence of TSH (■) and in presence of inhibitors (saccharide mixture + *N*-acetylneuraminic acid) and TSH (▲). Inhibitor concentration was 100 μg/ml. An ohmic relationship was observed in all cases up to about 40 mV and the results were identical for reverse polarity.

Table 2
Inhibiting effect of saccharides groups on the interaction between the GMO–GT_1 planar membranes and thyrotropin

Inhibitors[a]	Conductance ($\Omega^{-1}.cm^{-2}$)	% Inhibition
None	4.10^{-6} (11)[b]	0%
Lactose	$1.8.10^{-6}$ (8)	55%
Galactose	$1.4.10^{-6}$ (16)	65%
N-Acetylneuraminic acid	$1.1.10^{-6}$ (6)	73%
Glucose	$0.5.10^{-6}$ (12)	87%
N-acetylgalactosamine	$0.46.10^{-6}$ (8)	89%
Saccharide mixture + *N*-acetylneuraminic acid	$0.37.10^{-6}$ (8)	91%

[a] Inhibitor concentration 100 μg/ml
[b] No. experiments in brackets

clearly show that the hydrophilic part of GT_1 included in a lipid bilayer behaves as a thyrotropin receptor. The observed penetration of the TSH hormone in the membrane model suggests that the presumed presence of a glycolipid component in the TSH-receptor structure [15] should contribute to the functional transmission of the TSH message to the thyroid cell machinery. It is supposed therefore that receptor should have a specific carbohydrate sequence similar to the saccharide moiety of the GT_1 ganglioside.

Acknowledgements

We thank (M.D.) IRSIA (Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture) for financial support.

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