THE STRUCTURE AND FUNCTION OF GLYCOPROTEIN HORMONE RECEPTORS: GANGLIOSIDE INTERACTIONS WITH HUMAN CHORIONIC GONADOTROPIN

George Lee,[1] Salvatore M. Aloj,[1,2] Roscoe O. Brady,[3] and Leonard D. Kohn[1]

[1] Section on Biochemistry of Cell Regulation, Laboratory of Biochemical Pharmacology, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20014, U.S.A.

[2] Centro di Endocrinologia ed Oncologia Sperimentale C.N.R., Naples, Italy

[3] Developmental and Metabolic Neurology Branch, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health

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SUMMARY: Gangliosides inhibit the binding of ^{125}I-labeled human chorionic gonadotropin to rat testes membranes. The inhibition is the result of an interaction between the hormone and the ganglioside rather than the membrane and ganglioside, and the interaction with the ganglioside can be detected by fluorescence spectroscopy. In both the binding inhibition and fluorescence studies, human chorionic gonadotropin recognizes an oligosaccharide sequence on the ganglioside molecule distinct from the sequence recognized by thyrotropin.

In a previous report (1), we showed that gangliosides could inhibit ^{125}I-labeled thyrotropin binding to the thyrotropin receptors on bovine thyroid plasma membranes. This inhibition by gangliosides was critically altered by the number and location of the sialic acid residues within the ganglioside structure, the efficacy of inhibition having the following order: $G_{D1b} > G_{T1} > G_{M1} > G_{M2} = G_{M3} > G_{D1a}$.* The inhibition resulted from the interaction of thyrotropin (TSH)* and gangliosides, rather than from the interaction of membrane and gangliosides. In this regard, fluorescence studies showed that the ganglioside-thyrotropin interaction resulted in a distinct conformational change in the TSH molecule

* Abbreviations: TSH, thyrotropin; hCG, human chorionic gonadotropin: LH, luteinizing hormone; FSH, follicle-stimulating hormone; G_{M3}, N-acetylneuraminylgalactosylglucosylceramide; G_{M2}, N-acetylgalactosaminyl-[N-acetylneuraminyl]-galactosylglucosylceramide; G_{M1}, galactosyl-N-acetylgalactosaminyl-[N-acetylneuraminyl]-galactosylglucosylceramide; G_{D1a}, N-acetylneuraminyl-galactosyl-N-acetylgalactosaminyl-[N-acetylneuraminyl]-galactosylglucosylceramide; G_{D1b}, galactosyl-N-acetylgalactosaminyl-[N-acetyl-neuraminyl-N-acetylneuraminyl]-galactosylglucosylceramide; G_{T1}, N-acetylneuraminylgalactosyl-N-acetylgalactosaminyl-[N-acetylneuraminyl-N-acetylneuraminyl]-galactosylglucosylceramide; DNS, dimethylaminonaphtalenesulphonate.

and, more important, that there was a progression from a "noninhibitory conformation" to an "inhibitory conformation" which exactly paralleled the ability of the gangliosides to inhibit ^{125}I-labeled TSH binding.

As a result of these studies (1), we suggested that a ganglioside or ganglioside-like structure was a structural or functional component of the TSH receptor. We supported this suggestion with two findings. First, gangliosides more complex than G_{M3} were shown to be present in bovine thyroid membranes in much higher quantities than had been previously found in extraneural tissue (1). Second, the TSH receptor deficiency of a thyroid tumor which could not bind TSH was correlated with a deficiency in the ganglioside content of its membranes (2); this tumor has an adenylate cyclase activity unresponsive to TSH but normally responsive to prostoglandins and fluoride.

In the course of the above studies (1, 2), it was noted that the mechanism by which TSH transmitted its message to the cell had features in common with cholera toxin and that the B protein of cholera toxin had a peptide sequence not only in common with the β subunit of TSH (1, 3) but also with the β subunits of the other glycoprotein hormones: human chorionic gonadotropin (hCG)*, luteinizing hormone (LH)*, and follicle-stimulating hormone (FSH)* (3). This finding suggested that hCG, LH, and FSH might be similar to TSH and cholera toxin in their mechanism of receptor interaction but that their target organ specificity might result from their recognition of carbohydrate sequences on ganglioside or ganglioside-like receptor structures distinct from those recognized by TSH or cholera toxin (1, 4). In the present report we support the validity of this suggestion insofar as it extends to the structure and function of the hCG receptor.

MATERIALS AND METHODS

Rat testes membranes as well as ^{125}I-hCG were prepared as described by Bellisario and Bahl (5). The hCG was obtained from Dr. Robert E. Canfield, Columbia University College of Physicians and Surgeons, New York, through the Center for Population Research, NICHD, NIH. TSH was a bovine preparation prepared as described (6-9). ^{125}I-hCG binding to plasma membranes was assayed by a filtration technique already described (1, 8, 9). In addition to the agents tested for their ability to influence binding, binding assays contained in a

120-µl volume, 0.025 M Tris-acetate, pH 6.0, 0.6% bovine serum albumin, approximately 150,000 cpm (2×10^{-9} M) ^{125}I-labeled hCG, and 200 µg of membrane protein. The amount of plasma membranes used was within the linear range of binding when evaluated as a function of membrane protein concentration; the incubation was for 2 hours at 23°. To insure that the binding and the inhibition of binding measured in these assays were specific, control incubations containing 1.5×10^{-5} M unlabeled hCG or no membranes were included in each individual experiment.

Gangliosides G_{M2} and G_{D1a} were obtained as previously described (10). Gangliosides G_{M1}, G_{D1b}, and G_{T1} were isolated from commercial preparations (Supelco, Inc., Bellefonte, Pennsylvania) by preparative thin-layer chromatography (11). Each ganglioside used in these experiments was at least 99% pure after rechromatography (11). Gangliosides were quantitated by their sialic acid content using a micromodification of the resorcinol method of Svennerholm (12).

Dimethylaminonaphtalenesulphonate (DNS)* conjugates of hCG were prepared as previously described for bovine growth hormone (13). Unreacted dye was separated by filtration through a Sephadex G-25 (superfine) column (0.5 cm x 15 cm) eluted with 0.02 M glycine-NaOH, pH 8.6. The conjugate solution was finally dialyzed overnight against 0.02 M Tris-acetate, pH 8.2. The number of bound DNS molecules per mole of hCG was 1.1. Fluorescence measurements were carried out with a Turner model 210 spectrofluorometer. Samples for fluorescence studies contained 50 µl of the hCG-DNS conjugate and 1.45 ml of 0.02 M Tris-acetate, pH 7.5; absorption was within 0.1 absorbance unit at the excitation wavelength (340 nm).

RESULTS

Gangliosides inhibit ^{125}I-labeled hCG binding to rat testicular membranes, the efficacy of the inhibition being related to the oligosaccharide structure of

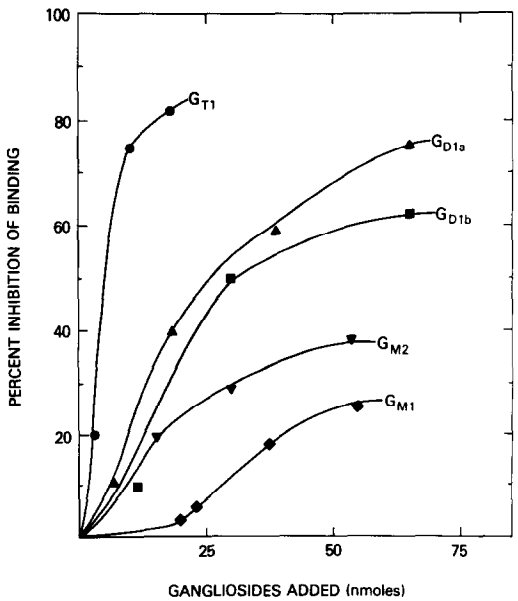

FIG. 1. *Ganglioside inhibition of ^{125}I-hCG binding to rat testes membranes. Binding was for 2 hours at 23° in 0.02 M Tris-acetate, pH 6.0.*

the ganglioside (Fig. 1). It was especially notable in this study that, after G_{T1}, one of the most effective inhibitors of hCG binding was G_{D1a}. This same ganglioside was the least effective inhibitor of TSH binding to thyroid plasma membranes (1) and was an ineffective inhibitor of cholera toxin binding to fat cell membranes (14, 15).

These experiments were performed using binding conditions similar to those previously determined to be optimal for TSH binding to thyroid plasma membranes, i.e., 0.02 M Tris-acetate, pH 6.0. The assay conditions used in these experiments did not significantly alter the specificity of hCG binding by comparison to previous studies of hCG binding which were performed using assay conditions having both a higher salt content and a higher pH: 0.05 M Tris-HCl, pH 7.2, containing 1 mM $CaCl_2$ (5). Thus, under both conditions unlabeled hCG caused

TABLE I. *Ganglioside inhibition of ^{125}I-hCG binding using assay conditions having both a higher pH and a higher salt content*

Ganglioside (30 nmol added)	Assay conditions: 0.02 M Tris-acetate, pH 6.0 [a]	Assay conditions: 0.05 M Tris-Cl, pH 7.2, containing 1 mM $CaCl_2$ [b]
	% inhibition of binding	
G_{D1a}	55	35
G_{D1b}	50	18
G_{M2}	27.5	9
G_{M1}	13.5	0

[a] Data taken from Fig. 1.

[b] Assay conditions were identical save for the change noted in buffer.

a 50% inhibition of ^{125}I-hCG binding to rat testes membranes at 100-fold lower concentrations than unlabeled bovine TSH. When ganglioside inhibition of ^{125}I-hCG binding was evaluated at this higher pH and at these higher salt concentrations, however, differences in ganglioside specificity were amplified

(Table I). Thus, although the ability of gangliosides to inhibit is lowered in these conditions, the inhibitory effect of G_{D1a} becomes more pronounced relative to G_{D1b}, G_{M2}, and G_{M1}.

TABLE II. *Effect of preincubation of membrane and ganglioside on inhibition of ^{125}I-hCG binding to rat testes membranes* [a]

Experiment	Preincubation components	% Inhibition
1	None	84
2	Membranes + ^{125}I-hCG	86
3	Ganglioside + ^{125}I-hCG	84
4	Ganglioside + membranes without centrifugation before assay	84
5	Ganglioside + membranes followed by centrifugation before assay	11

[a] In the control experiment where no preincubation was performed (experiment 1), all components (membranes, gangliosides, and ^{125}I-hCG) were added within 10 seconds, mixed, and incubated for a total of 2 hours prior to filtration. In experiments 2, 3, and 4, the noted components were preincubated in assay buffer for 15 minutes before the missing component, ganglioside, membranes, and ^{125}I-hCG, respectively, were added; the binding assay then proceeded for 2 hours before filtration. In experiment 5, after the ganglioside and membranes were preincubated for 15 minutes, the mixture was centrifuged at 12,000 x g for 15 minutes to sediment the membranes. The membranes were then resuspended in buffer, and the missing component, ^{125}I-hCG was added. The ganglioside preparation used in these experiments was a mixed preparation (from bovine brain) containing 47% G_{D1a}, 25% GT1, 16% G_{D1b}, and 12% G_{M1}; 70 nmol were added. In addition to serving as a control for experiment 5, experiment 2 shows that gangliosides can "chase" bound hCG off the membrane. Preincubation and binding was at 23°.

As in the case of the ganglioside inhibition of TSH binding to thyroid plasma membranes, the ganglioside inhibition of hCG binding to testes membranes was the result of the interaction of the ganglioside with the hormone rather than of an interaction with the membranes. Evidence for this conclusion came from the following experiments. First, there was no significant inhibition of hCG binding when gangliosides were preincubated with the membranes prior to incubation with the ^{125}I-hCG (Table II). Second, preincubation of ^{125}I-hCG with

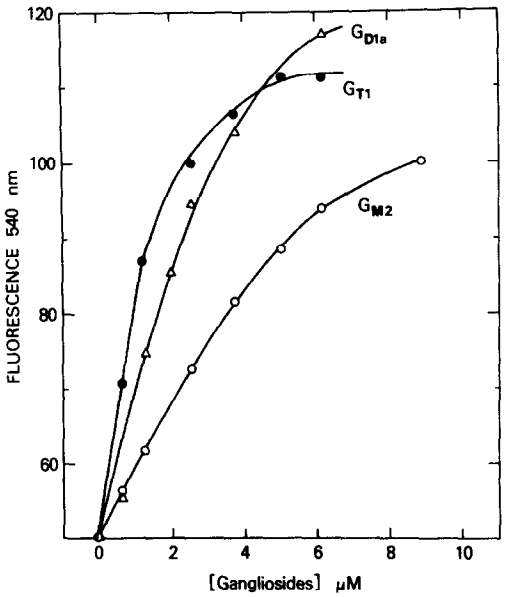

FIG. 2. *Effect of gangliosides on the fluorescence of DNS-hCG in 0.02 M Tris-acetate, pH 7.5, at 21°. The final concentration of hCG-DNS is 2 μM; excitation was at 340 nm.*

gangliosides resulted in a change in the elution pattern of the ^{125}I-hCG when it was subjected to gel filtration chromatography; in contrast, preincubation of ^{125}I-hCG and gangliosides in the presence of a 1,000-fold excess of unlabeled hCG prevented the altered elution pattern of the ^{125}I-hCG. Last, fluorescence studies demonstrated that gangliosides interact with hCG. This interaction is associated with a large increase of the emission intensity of DNS covalently linked to the hormone (Fig. 2). As in the case of the interaction of TSH with gangliosides (1), the magnitude of the fluorescence changes depends on the oligosaccharide structure of the gangliosides tested (Fig. 2) and reflects the efficacy with which the ganglioside inhibits binding of ^{125}I-hCG to the target organ membranes.

DISCUSSION

The present report suggests that hCG, like TSH, interacts with a receptor which has a ganglioside or ganglioside-like structure and that, like TSH and

cholera toxin, hCG undergoes a change in conformation upon interacting with its receptor structure. In addition, the report suggests that the oligosaccharide moiety of the hCG receptor structure is distinct from the oligosaccharide moiety on the TSH receptor since the ganglioside inhibition of hCG binding to rat testicular membranes exhibits a different specificity ($G_{T1} > G_{D1a} > G_{D1b} > G_{M2} > G_{M1}$) than the ganglioside inhibition of TSH binding to thyroid plasma membranes ($G_{D1b} > G_{T1} > G_{M1} > G_{M2} > G_{D1a}$). Most dramatic in this regard is the effectiveness of G_{D1a} as an inhibitor of hCG binding to rat testes membranes in contrast to its ability to inhibit TSH binding to thyroid membranes. Thus, the most effective inhibitors of hCG binding, G_{T1} and G_{D1a}, have a terminal sialic acid on the oligosaccharide chain, whereas the most effective inhibitors of TSH binding, G_{T1} and G_{D1b}, have a disialyl group linked to the internal galactose residue (1).

Since these data suggest that ganglioside or ganglioside-like structures are important determinants of the specificity of TSH and hCG interactions with their respective target organs, studies concerning the content and biosynthesis of gangliosides in the plasma membranes of the thyroid, testes, and other glycoprotein hormone sensitive target organs have been initiated. In addition, current experiments are aimed at [i] further correlating the extent of inhibition by a particular ganglioside with the conformational change that the ganglioside induces; [ii] determining the relationship of the conformational change to target organ specificity and hormone message propagation; and [iii] evaluating the possibility (suggested by the data in Table I) that alterations in salt or ion concentrations at the site of the hormone interaction with its ganglioside or ganglioside-like receptor might further amplify the specificity differences integral to target organ and hormonal specificity which are suggested in this report.

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