THE STRUCTURE AND FUNCTION OF GLYCOPROTEIN HORMONE RECEPTORS: GANGLIOSIDE INTERACTIONS WITH LUTEINIZING HORMONE

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Received June 6,1977

SUMMARY: Gangliosides inhibit the binding of ^{125}I-labeled luteinizing hormone to rat testis 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 ganglioside can be detected by fluorescence spectroscopy. In both the binding inhibition and fluorescence studies, luteinizing hormone recognizes an oligosaccharide sequence on the ganglioside molecule distinct from that of thyrotropin, human chorionic gonadotropin, and cholera toxin.

In previous reports we have suggested that the thyrotropin (TSH)* receptor on the thyroid plasma membrane is a complex containing both glycoprotein and ganglioside components (1); that the properties of the TSH receptor are derived from each of these components (1-4); that the functional transmission of the TSH message to the cell machinery requires the presence of both components (1-6); and that the role of the gangliosides in transmitting the hormonal message is analogous to their role in transmitting the message of cholera toxin to cells exposed to this bacterial product (1-12). In the course of these studies the existence of sequence analogies between cholera toxin, TSH, LH, hCG, and FSH were demonstrated (9, 13). These data (1-13) raised the following possibilities: all glycoprotein hormones have a similar receptor structure and a similar mechanism of receptor interaction; each target organ has in its receptor a specific or unique carbohydrate sequence, i.e., one which is different from that on other

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

ISSN 0006-291X

target organs; the interaction of the appropriate hormone with this oligosaccharide results in a conformational change in the hormone such that its α subunit is placed in a position favored for membrane interaction and adenylate cyclase stimulation in that particular target tissue.

A recent study of ganglioside inhibition of hCG binding to testis membranes (14) provided preliminary support for these hypotheses. Additional support is provided by the present report which examines (i) the ability of various gangliosides to inhibit LH binding to rat testis membranes, (ii) the nature of the ganglioside-LH interaction, and (iii) the ganglioside composition of rat testis membranes.

MATERIALS AND METHODS

Rat testis membranes were prepared as previously described (14, 15). Bovine LH was a gift of Dr. John Pierce, University of California at Los Angeles; ^{125}I-LH was prepared by adapting procedures used to iodinate TSH (16). ^{125}I-LH binding to rat testis membranes was assayed by a filtration technique already described (7, 16, 17). In addition to the agents tested for their ability to influence binding, the assay contained in a 100-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 LH, and 7 μg of membrane protein. The amount of plasma membranes used was within the linear phase of binding when evaluated as a function of membrane protein concentration; the incubation was carried out at 0° for 2 hours. 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 LH or no membranes were included in each individual assay.

Gangliosides G_{M1}, G_{M2}, G_{D1a}, G_{D1b}, and G_{T1} were obtained as previously described (7, 8, 14, 18, 19). Each ganglioside used in these experiments was at least 99% pure after rechromatography. Gangliosides were quantitated by their sialic acid content using a micromodification of the resorcinol method of Svennerholm (20).

Gangliosides were extracted from rat testis membranes by the method of Yu and Ledeen (21). Thin-layer chromatography and quantitation of sialic acid were carried out as previously described (19).

Fluorescence measurements were carried out at 25° ± 0.10° using the methodology previously described for studies of ganglioside-TSH interactions (7). LH concentrations are based on their absorbance at 276.5 nm using an E_M = 12,100 liter/mole per cm. This extinction coefficient was calculated from the amino acid composition of LH (22) assuming a contribution of 1,500 for each tyrosine (23, 24) and 145 for each disulfide bond (24). Protein was assayed using a colorimetric procedure (25) including crystalline serum albumin as the standard.

RESULTS

Gangliosides Inhibit LH Binding — Gangliosides inhibit the binding of ^{125}I-labeled LH to rat testis membrane preparations (Fig. 1). Their ability to inhibit LH binding ($G_{T1} > G_{D1b} >> G_{D1a} > G_{M1} > G_{M2}$) is distinct from their

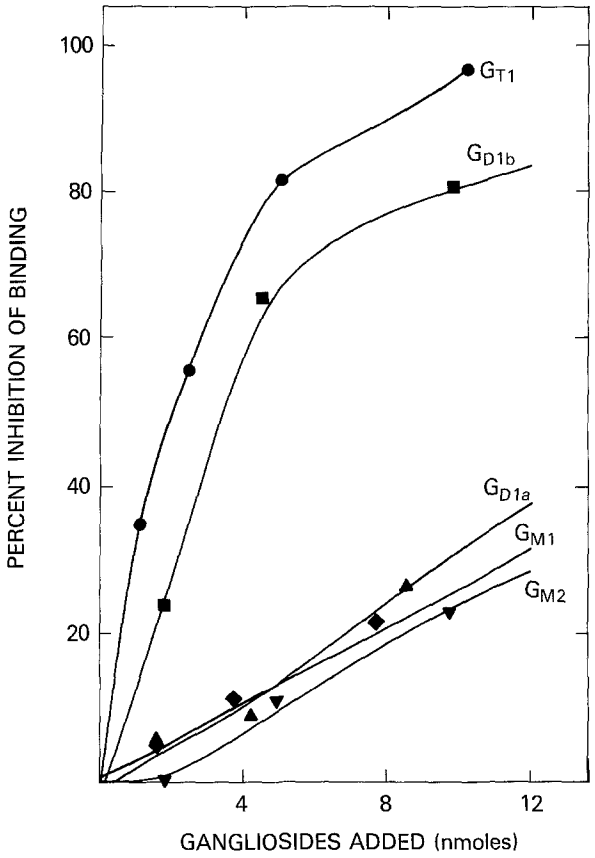

FIG. 1. Ganglioside inhibition of ^{125}I-LH binding to rat testis membranes. Binding was for 2 hours, at 0°, and in 0.025 M Tris-acetate, pH 6.0.

ability to inhibit TSH binding to bovine thyroid membranes ($G_{D1b} > G_{T1} > G_{M1} > G_{M2} > G_{D1a}$) (7, 11, 12) and hCG binding to rat testis membranes ($G_{D1b} > G_{D1a} > G_{D1b} > G_{M2} > G_{M1}$) (14). In addition, the pattern of inhibition is remarkable in that the gangliosides can be grouped into two classes, one with high inhibitory effect (G_{T1}, G_{D1b}) and one with low inhibitory effect (G_{D1a}, G_{M1}, G_{M2}); this is quite different from the ganglioside inhibition pattern of TSH and hCG binding which appears more evenly spread (7, 14). Ganglioside inhibition of LH binding shares a greater similarity with ganglioside inhibition of TSH binding to thyroid membranes (7) than with the inhibition of hCG binding to the testis membranes (14). For example, G_{D1a} is a poor inhibitor of LH and TSH binding and

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

Experiment	Preincubation components	% inhibition
1	None	82
2	Membranes + ^{125}I-LH	82
3	Ganglioside + ^{125}I-LH	81
4	Ganglioside + membranes without centrifugation before assay	82
5	Ganglioside + membranes followed by centrifugation before assay	14

[a] In the control experiment where no preincubation was performed (experiment 1), all components (membranes, gangliosides, and ^{125}I-LH) 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-LH, 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-LH, was added. The ganglioside preparation used in these experiments was a mixed preparation (from bovine brain) containing 47% G_{D1a}, 25% G_{T1}, 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 LH off the membrane. Preincubation and binding were at 0°.

a potent inhibitor of hCG binding. Last, comparable inhibition of LH binding is achieved by lower concentrations of gangliosides than are necessary to demonstrate the inhibition of TSH and hCG binding [Fig. 1 and (7, 14)].

Ganglioside-LH Interaction — ^{125}I-LH binding is not inhibited by gangliosides when membranes are preincubated with gangliosides and gangliosides are subsequently removed by centrifugation (Table I), i.e., ganglioside inhibition of LH binding does not appear to be the result of a ganglioside-membrane interaction. In contrast, ^{125}I-LH is more rapidly eluted from Sephadex G-100 columns (data not shown) after being preincubated with mixed brain gangliosides. The implication of this experiment, that gangliosides can interact with LH, is

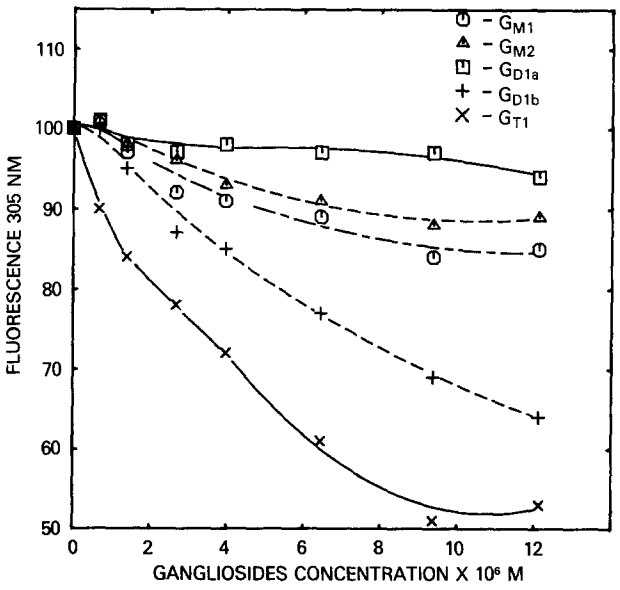

FIG. 2. Effect of gangliosides on the fluorescence of LH in 0.02 M Tris-acetate, pH 7.5, at 21°. The final concentration of LH is 2 μM.

confirmed by fluorescence studies (Fig. 2) which show that the interaction of the gangliosides with LH is similar to the interaction of the gangliosides with TSH or hCG, i.e., as is the case for TSH (7) and hCG (14), there is a good correlation between changes in hormone fluorescence caused by individual gangliosides and the relative efficacy of inhibition.

Ganglioside Content of Rat Testis Membranes — Rat testis membranes contain 2.5 nmol of lipid-bound sialic acid per mg membrane protein, of which approximately 15% is G_{M3} and the remainder is higher-order gangliosides (Fig. 3). The higher-order homologs include gangliosides with the chromatographic properties of authentic G_{M2}, G_{D1a}, G_{D1b}, and G_{T1}. The ganglioside pattern of rat testis membranes is distinct from that of both rat (5) and bovine thyroid membranes (7) similarly extracted and analyzed.

DISCUSSION

The present report demonstrates that higher-order gangliosides exist in rat

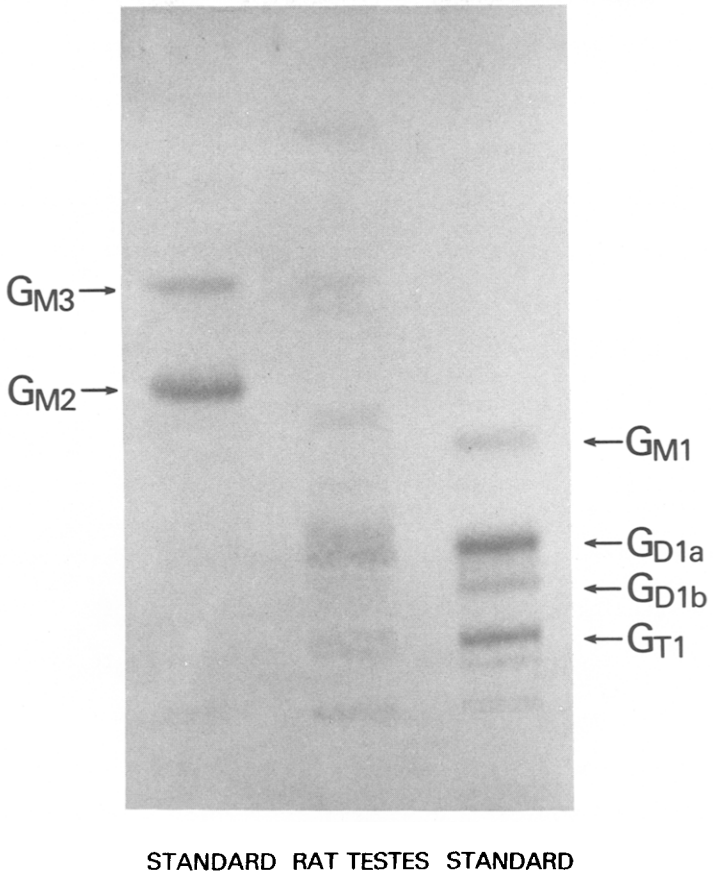

FIG. 3. Thin-layer chromatogram of gangliosides from rat testis membranes. Preparation of rat testis membranes and extraction of gangliosides were performed as described (see "Materials and Methods"). Visualization is by resorcinol reagent (see "Materials and Methods").

testis membrane preparations; that gangliosides inhibit LH binding to testis membranes; that the efficacy of inhibition by each individual ganglioside correlates with its ability to cause a distinct perturbation in the intrinsic fluorescence of the LH molecule; and that the ability of gangliosides to inhibit LH binding is distinct from the ability of gangliosides to inhibit hCG binding to the same membrane preparations or TSH binding to thyroid membrane preparations. In short, the present study of the LH receptor presents evidence analogous to that which led to the suggestion that a ganglioside is a component of the TSH receptor (1-9, 11, 12) and that there exists within the receptor

structure a critical oligosaccharide determinant which, by imposing a unique conformational change in the hormone molecule, allows appropriate propagation of the hormonal message (1-9, 11, 12, 14). This report thus supports the hypothesis (i) that there is a structural relationship among the receptors for the glycoprotein hormones and (ii) that the oligosaccharide determinant in the receptor structure is a key element in the target organ specificity exhibited by glycoprotein hormones (14).

Membrane preparations from different target organs appear to have distinctive ganglioside patterns on thin-layer chromatograms [Fig. 3 and (5, 7)] and have different amounts of the identifiable higher-order ganglioside components. Nevertheless, each membrane preparation contains ganglioside components with the chromatographic mobility of G_{D1b} and G_{T1}, i.e., the gangliosides most effective as inhibitors of binding in each case studied, LH, hCG, and TSH (7, 14). The question therefore arises as to how gangliosides contribute to target organ specificity. Several possibilities exist. First, there can be gangliosides which are unique to each target tissue, which are the key determinants, and which are structurally related to G_{D1b} and G_{T1}. This possibility has been raised by the identification of a minor component of the ganglioside fraction in bovine thyroid tissue with an even better ability to inhibit TSH binding (on a molar basis) than G_{D1b} (26). Second, minor changes in linkages between the oligosaccharide sugar moieties or even sugar substitutions (27, 28) could influence ganglioside-hormonal interactions and target organ specificity. These and other possibilities, i.e., differences in interactions between glycoprotein and glycolipid membrane components and differences in other membrane components which could influence these interactions or membrane fluidity, are under investigation in order to define the role of the ganglioside in the message transmission process and in adenylate cyclase stimulation.

REFERENCES

1. Meldolesi, M. F., Fishman, P. H., Aloj, S. M., Ledley, F. D., Lee, G., Bradley, R. M., Brady, R. O., and Kohn, L. D. (1977) *Biochem. Biophys. Res. Commun.* 75, 581-588.

2. Aloj, S. M., Kohn, L. D., Lee, G. , and Meldolesi, M. F. (1977) *Biochem. Biophys. Res. Commun.* 74, 1053-1059.
3. Tate, R. L., Holmes, J. M., Kohn, L. D., and Winand, R. J. (1975) *J. Biol. Chem.* 250, 6527-6533.
4. Tate, R. L., Winand, R. J., and Kohn, L. D. (1976) *in* Robbins, J., and Braverman, L. E. (eds.): *Thyroid Research*, New York, American Elsevier Publishing Co., pp. 57-60.
5. Meldolesi, M. F., Fishman, P. H., Aloj, S. M., Kohn, L. D., and Brady, R. O. (1976) *Proc. Natl. Acad. Sci. U.S.A.* 73, 4060-4064.
6. Winand, R. J., and Kohn, L. D. (1975) *J. Biol. Chem.* 250, 6534-6540.
7. Mullin, B. R., Fishman, P. H., Lee, G., Aloj, S. M., Ledley, F. D., Winand, R. J., Kohn, L. D., and Brady, R. O. (1976) *Proc. Natl. Acad. Sci. U.S.A.* 73, 842-846.
8. Mullin, B. R., Aloj, S. M., Fishman, P. H., Lee, G., Kohn, L. D., and Brady, R. O. (1976) *Proc. Natl. Acad. Sci. U.S.A.* 73, 1679-1683.
9. Ledley, F. D., Mullin, B. R., Lee, G., Aloj, S. M., Fishman, P. H., Hunt, L. T., Dayhoff, M. O., and Kohn, L. D. (1976) *Biochem. Biophys. Res. Commun.* 69, 852-859.
10. Bennett, V., and Cuatrecasas, P. (1977) *in* Cuatrecasas, P. (ed.): *The Specificity and Action of Animal, Bacterial, and Plant Toxins*, New York, Chapman and Hall, in press.
11. Kohn, L. D., Lee, G., Grollman, E. F., Friedman, R. M., Ledley, F. D., Meldolesi, M. F., Mullin, B. R., and Aloj, S. M. (1977) *in* Harmon, R. E. (ed.): *Symposium on Cell Surface Carbohydrate Chemistry, San Francisco, California, Centennial Meeting of the American Chemical Society*, in press.
12. Kohn, L. D. (1977) *in* Quagliariello, E. (ed.): *Horizons in Biochemistry and Biophysics*, Reading, Massachusetts, Addison-Wesley Publishing Co., Vol. 3, pp. 123-159.
13. Kurosky, A., Markel, D. E., Peterson, J. W., and Fitch, W. M. (1977) *Science* 195, 299-301.
14. Lee, G., Aloj, S. M., Brady, R. O., and Kohn, L. D. (1976) *Biochem. Biophys. Res. Commun.* 73, 370-377.
15. Bellisario, R., and Bahl, O. P. (1975) *J. Biol. Chem.* 250, 3837-3844.
16. Tate, R. L., Schwartz, H. I., Holmes, J. M., Kohn, L. D., and Winand, R. J. (1975) *J. Biol. Chem.* 250, 6509-6515.
17. Amir, S. M., Carraway, T. F., Jr., Kohn, L. D., and Winand, R. J. (1972) *J. Biol. Chem.* 248, 4092-4100.
18. Fishman, P. H., McFarland, V. W., Mora, P. T., and Brady, R. O. (1972) *Biochem. Biophys. Res. Commun.* 48, 48-57.
19. Fishman, P. H., Brady, R. O., Bradley, R. M., Aaronson, S. A., and Todaro, G. J. (1974) *Proc. Natl. Acad. Sci. U.S.A.* 71, 298-301.
20. Svennerholm, L. (1957) *Biochim. Biophys. Acta* 24, 604-615.
21. Yu, R. K., and Ledeen, R. W. (1972) *J. Lipid Res.* 13, 680-686.
22. Pierce, J. G., Liao, T. H., Howard, S. M., Shome, B., and Cornell, J. S. (1971) *Recent Prog. Horm. Res.* 27, 165-212.
23. Brandts, J. F., and Kaplan, L. J. (1973) *Biochemistry* 12, 2011-2024.
24. Edelhoch, H. (1967) *Biochemistry* 6, 1948-1954.
25. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.* 193, 265-275.
26. Mullin, B. R., Pacuszka, T., Lee, G., Kohn, L. D., Brady, R. O., and Fishman, P. H. (1977) *Science*, in press.
27. Lagrou, A., Hilderson, H. J., De Wolf, M., and Dierick, W. (1974) *Arch. Int. Physiol. Biochim.* 82, 733-736.
28. Van Dessel, G., Lagrou, A., Hilderson, H. J., and Dierick, W. (1976) *Arch. Int. Physiol. Biochim.* 84, 674-676.