THE BINDING OF THYROTROPIN TO LIPOSOMES CONTAINING GANGLIOSIDES

Salvatore M. Aloj[1,2], Leonard D. Kohn[1], George Lee[1], and Maria F. Meldolesi[1,2]

[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 del C.N.R., Naples, Italy

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SUMMARY: Multilamellar liposomes containing mixed brain gangliosides specifically bind ^{125}I-labeled bovine thyrotropin. Ganglioside-free liposomes do not bind the hormone. The amount of hormone bound correlates with the molar ratio gangliosides : phospholipid. The binding of ^{125}I-labeled thyrotropin to gangliosides containing liposomes resembles the binding to thyroid plasma membranes; it is characterized by relatively high affinity, is competed for by the cold hormone, and is decreased by increasing ionic strength and temperature.

We have recently suggested that a ganglioside or a ganglioside-like structure may be an important component of the thyrotropin (TSH)* receptor on plasma membranes of both thyroid and retro-orbital tissue (1). The suggestion was based on the following evidence: Gangliosides interact with TSH as shown by physical techniques such as fluorescence spectroscopy and analytical ultracentrifugation and inhibit the binding of TSH to plasma membranes (1). The efficacy of binding inhibition depends on the number and location of sialic acid residues on the ganglioside molecule (1). Thyroid membranes contain gangliosides more complex than G_{M3} in an amount unusual for extraneural tissue (1). A transplantable rat thyroid tumor with abnormal TSH binding lacks the more complex gangliosides present in the normal thyroid (2).

A more direct approach to prove that gangliosides can behave as receptors for TSH would be to incorporate gangliosides into multilamellar smectic mesophases of phospholipids and cholesterol (liposomes) and show that such model

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* Abbreviations: TSH, thyrotropin; hCG, human chorionic gonadotropin; G_{M2}, N-acetylgalactosaminyl-[N-acetylneuraminyl]-galactosylglucosylceramide; 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.

membranes share with the authentic thyroid plasma membranes some of the binding properties for ^{125}I-TSH. Liposomes containing gangliosides have been used to study the binding of plant lectins (3) as well as cholera toxin (4). This report describes the preparation of ganglioside-containing liposomes which do bind TSH specifically, with relatively high affinity, and with properties similar to those of authentic thyroid plasma membranes.

MATERIALS AND METHODS

Synthetic dipalmitoyl DL-α-phosphatidylcholine was obtained from the Sigma Chemical Company. Cholesterol esters (palmitate) (batch No. 9668) and bovine brain gangliosides (batch No. 7318) were obtained from the Nutritional Biochemicals Division of ICN Life Sciences Group. The latter are essentially free from polar lipids other than gangliosides. The relative proportion of each of the major individual gangliosides in this preparation are as follows, as determined by analytical thin-layer chromatography: G_{M2}* = 3%, G_{M1} = 19%, G_{D1a} = 50%, G_{D1b} = 8%, and G_{T1} = 12%. Bovine TSH and ^{125}I-TSH were prepared as previously described (5, 6). All other chemicals were reagent grade.

Preparation of liposomes. The procedure described by Kinsky et al. (7) has been used with some modifications. Dipalmitoyl phosphatidylcholine and cholesterol in a 2 : 1 molar ratio were dissolved in chloroform methanol (2 : 1). In a typical preparation 2 ml of this mixture were placed into a pear-shaped flask and dried under a stream of N_2 while the flask was gently shaken. To the lipid film thus obtained, mixed brain gangliosides in 2 ml 0.02 M Tris-acetate, pH 7.0, containing 0.1 M KCl, were added (molar ratio of gangliosides : phospholipids ≅ 0.05-0.1), and the flask was gently shaken overnight at 4°. Small glass beads (3 mm diameter) were added to the flask which was then vortexed for 5-10 minutes until the lipid suspension had become homogeneous. The liposomes were washed 3 times by dilution into a 5-fold volume of 0.02 M Tris-acetate, pH 7.0, followed by centrifugation at 7,000 rpm (Sorvall RC2, SS34 rotor). The final liposome pellet was resuspended into 2 ml of 0.02 M Tris-acetate, pH 7.0, and stored at 0-4°. Fifty µl of an 8-fold dilution of the liposome suspension were used in the binding assay. "Control liposomes" were prepared similarly, only the addition of gangliosides was omitted. An estimate of the number of particles contained in 50 µl of liposomes was obtained by using a Coulter counter model ZB1 with lower and upper thresholds set at 10 µm and 100 µm, respectively; an average of 5 x 10^5 particles were counted.

^{125}I-TSH binding to liposomes was assayed by the filtration technique previously described for thyroid plasma membranes (1, 8). The amount of liposomes used was within the linear phase of binding when the latter was evaluated as a function of liposome concentration.

RESULTS

Liposomes containing gangliosides bind ^{125}I-labeled TSH (Fig. 1 and Table I), and the amount of hormone bound appears to be linearly related to the molar ratio of gangliosides to phosphatidyl choline (Table I). Liposomes lacking gangliosides, i.e., containing only phosphatidyl choline and cholesterol, show

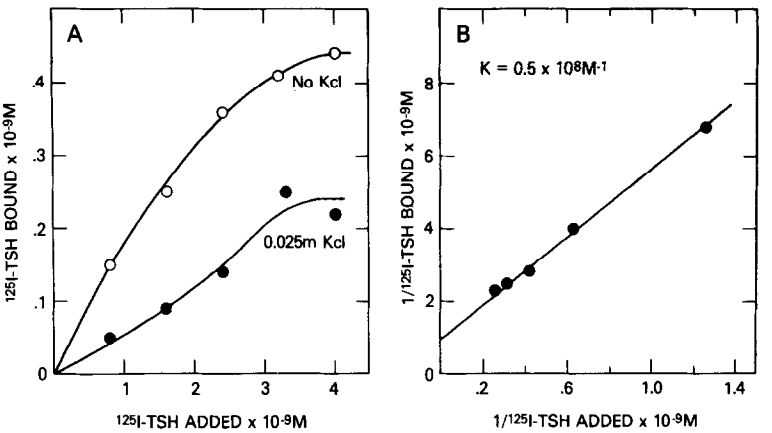

FIG. 1. A. Binding of ^{125}I-TSH to liposomes containing mixed brain gangliosides in a molar ratio to phosphatidyl choline = 0.05 (see "Materials and Methods"). Incubation was for 1 hour in 0.02 M Tris-acetate, pH 6.0, containing 0.5% bovine serum albumin in the absence (O) and in the presence (●) of 0.025 M KCl. The temperature was 0°. The ^{125}I-TSH bound to "control liposomes," i.e., liposomes containing only phosphatidyl choline and cholesterol, has been subtracted. B. Double reciprocal plot of the "No KCl" data of panel A.

TABLE I. *Binding of ^{125}I-TSH to liposomes containing different gangliosides : phosphatidylcholine molar ratios* [a]

^{125}I-TSH added cpm x 10^{-3}	[gangliosides] / [phosphatidylcholine]	^{125}I-TSH bound cpm x 10^{-3}
370	no gangliosides	2
370	0.05	41
370	0.075	58
370	0.1	84

[a] Binding assay conditions are identical to those reported in Fig. 1. No KCl was present.

negligible binding (Table I). Under the following experimental conditions, incubation in a 0.2 ml final volume for 1 hour at 0° in 0.02 M Tris-acetate, pH 6.0, containing 0.5% bovine serum albumin, about 5 x 10^5 particles (molar ratio

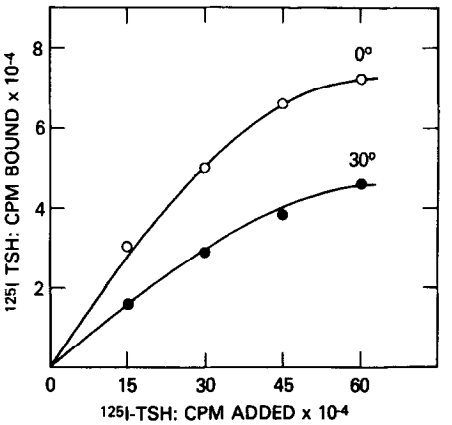

FIG. 2. The effect of temperature on the binding of ^{125}I-TSH to liposomes containing mixed brain gangliosides in a molar ratio to phosphatidyl choline = 0.075. Conditions are identical as for Fig. 1A; no KCl was added.

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gangliosides to phosphatidylcholine = 0.05) bind half-maximally at ~ 1.3×10^{-9} M ^{125}I-TSH (Fig. 1A). From a double reciprocal plot of the data (Fig. 1B), a binding constant of 0.5×10^{8} M^{-1} can be estimated. It is important to point out that this value is comparable to that previously reported for the "low affinity" receptor sites on thyroid plasma membranes (0.25×10^{8} M^{-1}) (6).

^{125}I-TSH binding to gangliosides containing liposomes is both salt- and temperature-dependent (Figs. 1A and 2). Thus, as has been previously reported for ^{125}I-TSH binding to thyroid plasma membranes (6), the binding to ganglioside-containing liposomes is reduced about 50% in 0.025 M KCl (Fig. 1A). Also in agreement with ^{125}I-TSH binding to thyroid plasma membranes (6), the binding of ^{125}I-TSH to the ganglioside-containing liposomes is greater at 0° than at 30° (Fig. 2). At 30° dipalmitoyl phosphatidylcholine, the phospholipid component of the liposomes, is still below the transition temperature which occurs around 42°; therefore, the differences in binding activity may not depend upon the conformational state of the phospholipid moiety of the liposomes and probably reflect the temperature dependence of TSH-ganglioside interaction.

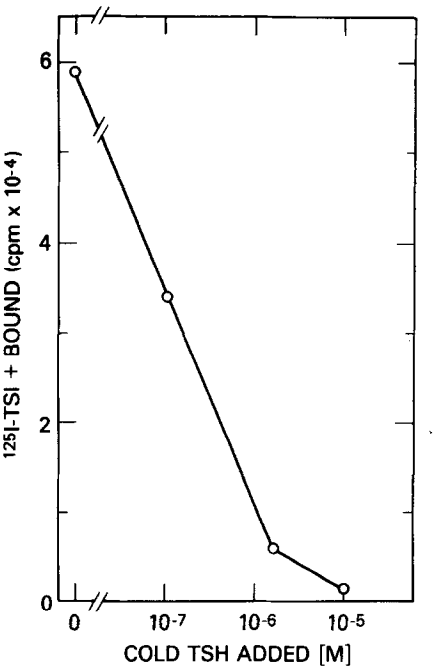

FIG. 3. The effect of unlabeled TSH on the binding of ^{125}I-TSH to liposomes containing gangliosides at 0°. The unlabeled TSH and the ^{125}I-TSH were added to the incubation mixtures simultaneously.

The binding of TSH to ganglioside-containing liposomes appears to be specific since it can be competed for by cold TSH (Fig. 3), but not by hCG*, bovine prolactin, insulin, or glucagon (data not shown). It is of interest that using the same TSH preparations and identical experimental conditions, inhibition of binding of labeled TSH to thyroid plasma membranes requires 5- to 10-fold higher concentrations of the cold hormone (6, 8-10).

DISCUSSION

In previous reports (1, 2) we have used several lines of indirect evidence to postulate a role for gangliosides as components of the receptors for glycoprotein hormones. In the present report we provide direct evidence that gangliosides can indeed provide receptor sites for ^{125}I-TSH which, when included in lipid bilayer model membranes, exhibit a salt and temperature dependence of binding and an affinity for the hormone comparable to that of receptors on

authentic thyroid plasma membranes. It is to be noted that these data do not exclude the possibility that other membrane components are necessary for both the binding of the hormone and the transmission of its message to the cell machinery. In this regard, liposomes containing a soluble TSH receptor glycoprotein isolated from bovine thyroid plasma membranes (9) also exhibit specific binding for ^{125}I-TSH (S. M. Aloj *et al.*, manuscript in preparation). It is possible that the glycoprotein receptor and gangliosides share common oligosaccharide moieties which are responsible for the specificity of TSH binding. It is also possible that glycoprotein and gangliosides may both be necessary for the plasma membrane to modulate its interaction with the hormonal effector. The system so far described will be especially useful if hormone-sensitive functional properties of plasma membranes, such as ion transport, can be reproduced by the liposomes containing the various modulators of the hormone-membrane interactions. In this regard, the report by Moss *et al.* (4), on the ability of choleragen to release trapped glucose from liposomes containing G_{M1}, is very useful and encouraging in view of the marked similarities in the structure and function of choleragen and the glycoprotein hormones (1, 10, 11).

In conclusion, this system should provide a useful model for investigating the role of gangliosides and other membrane constituents as receptors for glycoprotein hormones *in situ*.

REFERENCES

1. 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.* 76, 842-846.
2. Meldolesi, M. F., Fishman, P. H., Aloj, S. M., Kohn, L. D., and Brady, R. O. (1976) *Proc. Natl. Acad. Sci. U.S.A.* 76, 4060-4064.
3. Surolia, A., Bachhawat, B. K., and Podder, S. K. (1975) *Nature* 257, 802-804.
4. Moss, J., Fishman, P. H., Richards, R. L., Alving, C. R., Vaughan, M., and Brady, R. O. (1976) *Proc. Natl. Acad. Sci. U.S.A.* 73, 3480-3483.
5. Winand, R. J., and Kohn, L. D. (1970) *J. Biol. Chem.* 245, 967-975.
6. Tate, R. L., Schwartz, H. I., Holmes, J. M., Kohn, L. D., and Winand, R. J. (1975) *J. Biol. Chem.* 250, 6509-6515.
7. Kinsky, S. C., Haxby, J. A., Zopf, D. A., Alving, C. R., and Kinsky, C. B. (1969) *Biochemistry* 8, 4149-4158.

8. Amir, S. M., Carraway, T. F., Jr., Kohn, L. D., and Winand, R. J. (1973) *J. Biol. Chem.* 248, 4092-4100.
9. Tate, R. L., Holmes, J. M., Kohn, L. D., and Winand, R. J. (1975) *J. Biol. Chem.* 240, 6527-6533.
10. 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.
11. 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.