SEPARATION OF THE GLYCOPROTEIN AND GANGLIOSIDE COMPONENTS OF THYROTROPIN RECEPTOR ACTIVITY IN PLASMA MEMBRANES

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Received February 14,1977

SUMMARY: The two components of thyroid plasma membranes known to interact with thyrotropin, i.e., a glycoprotein with specific thyrotropin binding activity and the gangliosides of the thyroid membranes, are shown to segregate differently when membranes are solubilized with lithium diiodosalicylate. Individually examined, the interaction of each component with thyrotropin exhibits a different sensitivity to salts. The data suggest that the thyrotropin receptor on the thyroid membrane is a complex which is composed of both glycoprotein and ganglioside components and that its properties are derived from each component.

It has been reported previously that thyrotropin (TSH) receptors on bovine thyroid plasma membranes can be solubilized with lithium diiodosalicylate (1) and that tryptic digestion of the solubilized receptor preparation yields a 24,000 molecular weight receptor fragment which retains specific TSH binding activity (1, 2). Analysis of a purified preparation of this receptor fragment indicated that it was a glycoprotein containing 30% carbohydrate and 10% sialic acid by weight (1, 2). More recent studies indicated that gangliosides might be an important component of the thyrotropin receptor and that the role of gangliosides in transmitting the hormonal message to the cell machinery was analogous to their role in transmitting the message of cholera toxin to cells exposed to this bacterial product (3-10).

Abbreviations: TSH, thyrotropin; G_{M3}, N-acetylneuraminylgalactosylglucosylceramide; G_{M2}, N-acetylgalactosaminyl-[N-acetylneuraminyl]-galactosylglucosylceramide; G_{M1}, galactosyl-N-acetylgalactosaminyl-[N-acetylneuraminyl]-galactosylglucosylceramide; G_{D1a}, N-acetylneuraminylgalactosyl-N-acetylgalactosaminyl-[N-acetylneuraminyl]-galactosylglucosylceramide; LIS, lithium diiodosalicylate.

ISSN 0006-291X

TABLE I. ^{125}I-TSH binding and ganglioside content of intact bovine thyroid plasma membranes and of fractions obtained by "solubilizing" the membranes with lithium diiodosalicyate (LIS) [a]

	Total ^{125}I-TSH [b] binding activity		Total ganglioside [c] content		Total protein	
	cpm x 10^{-6}	%	nanomoles	%	mg	%
Intact membranes [d]	73	100	175	100	30	100
LIS supernatant (soluble receptor activity)	58	79	17	10	24	80
LIS pellet	15	21	149	85	4.5	15

[a] Solubilization used the procedure previously described (1, 2).

[b] Total binding activity is that obtained by multiplying the ^{125}I-TSH binding activity (in cpm) of an aliquot of the membrane, supernatant, or pellet preparations by the total volume of these preparations.

[c] Total gangliosides were extracted and purified from intact membranes and the LIS fractions as described in "Materials and Methods" and are expressed as nanomoles of sialic acid.

[d] Total binding activity of intact membranes was the same, using assay procedures developed for either plasma membrane or soluble receptor activity.

In the present study we report on the relative contribution of the glycoprotein and ganglioside components of thyroid plasma membranes to TSH receptor activity.

MATERIALS AND METHODS

Purified preparations of bovine TSH and ^{125}I-TSH were obtained as previously described (11-13) as were the thyroid plasma membranes (13, 14). Solubilization of the plasma membranes with lithium diiodosalicylate was at 23° for 1 hour (1, 2).

Gangliosides were extracted from the different preparations by a modification of the method of Yu and Ledeen (15). Briefly, the fractions were lyophilized, extracted with 20 ml of chloroform-methanol (2 : 1, v/v) at 37° for 30 minutes and re-extracted with 10 ml of chloroform-methanol (1 : 2, v/v) at 45° for 30 minutes. The combined extracts were chromatographed on DEAE-Sephadex (200 mg) (15). The ganglioside fraction was saponified in 0.6 ml of 0.2 M NaOH in chloroform-methanol (2 : 1, v/v) at 37° for 1 hour, neutralized with 30 µl of 4 M acetic acid, desalted on 0.5 g of Sephadex G-25 (16), and chromatographed on 100 mg of unisil (15). Thin-layer chromatography of gangliosides (16) and determination of ganglioside sialic acid (3) were done as previously described. Aganglioside membrane preparations were either the extracted residue from the above procedure or were preparations obtained by a technique described by Cuatrecasas (17). The aganglioside preparations were suspended in 0.1 M Tris-acetate, pH 6.0, by homogenization in a Potter-Elvjhem apparatus.

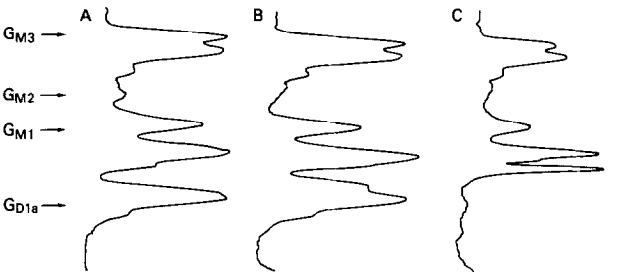

FIG. 1. Densitometric scans of a thin-layer chromatogram of gangliosides isolated from intact thyroid membranes (A), LIS pellet (B), and LIS supernatant (C). Gangliosides extracted from the two LIS fractions as well as the intact membranes (30 mg protein) described in Table I were chromatographed on thin-layer silica gel and detected with resorcinol reagent as described in "Materials and Methods." The thin-layer chromatogram was then scanned at 580 nm with a Zeiss spectrophotometric chromatogram scanner equipped with a chart recorder. The amounts of gangliosides applied to the chromatogram were 1/20th of the total for (A) and (B) and 1/5th of the total for (C). As presented, the direction of migration is from bottom to top; the migration of G_{M3}, G_{M2}, G_{M1}, and G_{D1a} was determined by cochromatography of a standard mixture of these gangliosides adjacent to each experimental sample. The ganglioside corresponding to G_{D1a} in fraction C migrated higher on this chromatogram, i.e., it is displaced upward by comparison to the G_{D1a} peaks in fractions B and C.

^{125}I-TSH binding to both intact plasma membranes and solubilized membrane preparations used procedures and incubation conditions detailed previously (1, 3, 13, 14). Fluorescence measurements were also carried out as previously described (3), and protein was assayed using a colorimetric procedure (18) including crystalline serum albumin as the standard.

RESULTS

When bovine thyroid membranes (30 mg protein) were homogenized with 0.1 M lithium diiodosalicylate (5 ml) and the homogenate was allowed to remain at room temperature for 1 hour, nearly 80% of the ^{125}I-TSH binding activity of the membranes was recovered in the supernatant solution after centrifugation for 30 minutes at 35,000 x g (Table I). The remainder of the ^{125}I-TSH binding activity was recovered in the pellet. This distribution of binding activity was in sharp contrast, however, to a 10 : 85 distribution in these fractions of the gangliosides present in thyroid membranes (Table I). Thin-layer chromatographic analysis of the gangliosides extracted from the membranes (Fig. 1A), from the pellet (Fig. 1B), and from the supernatant (Fig. 1C), indicated that the distribution of the individual ganglioside components in each fraction was proportional to

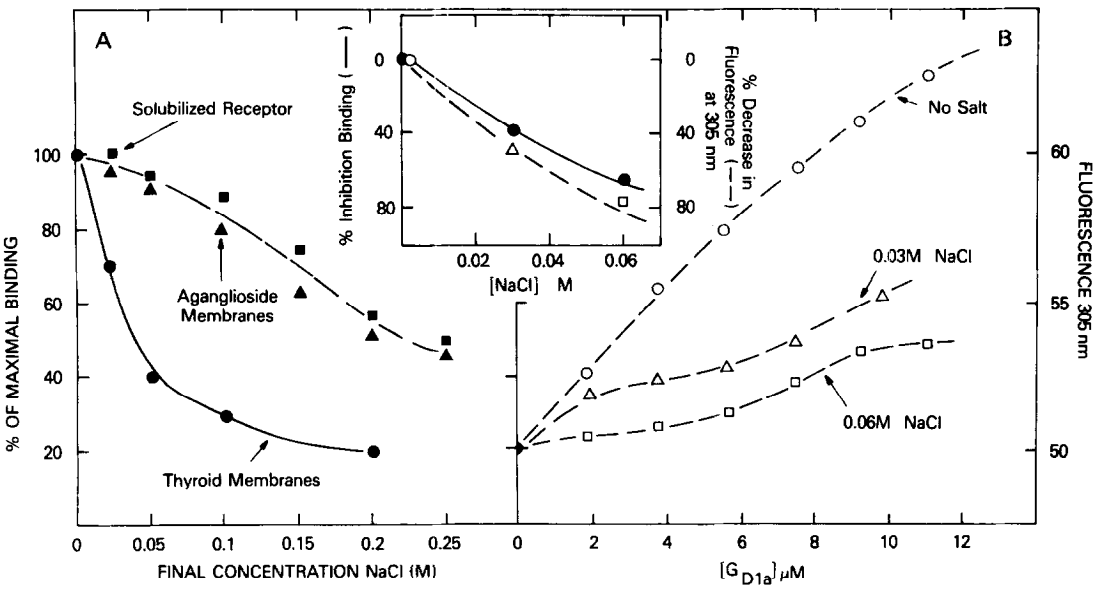

FIG. 2. (A) Salt (NaCl) inhibition of ^{125}I-TSH binding to thyroid plasma membranes (●), to the solubilized receptor activity of the supernatant solution obtained by treatment of these membranes with lithium diiodosalicylate (■), and to aganglioside thyroid plasma membranes (▲), i.e., membrane preparations which had been subjected to chloroform-methanol extraction procedures to remove membrane gangliosides (see "Materials and Methods"). (B) The effect of salt (NaCl) on the interaction of TSH with the ganglioside G_{D1a}. The insert relates the NaCl inhibition of membrane binding in Fig. 2A and the NaCl effect on TSH fluorescence in Fig. 2B. The data concerning the decrease in fluorescence from the 10 μM G_{D1a} data points (○, △, □) in Fig. 2B.

the total ganglioside distribution, i.e., no fraction appeared to be enriched in a particular ganglioside as a result of the procedure.

These results suggested that the ^{125}I-TSH binding activity of the glycoprotein receptor component present in the supernatant (1, 2) was an intrinsic property of this glycoprotein and did not require the presence of gangliosides. Support for this suggestion came from studies of the membrane residue after it had been subjected to the chloroform-methanol procedures used to extract the gangliosides. The delipidated or aganglioside residue retained 24% of the specific ^{125}I-TSH binding activity of the non-extracted membranes after the extraction procedure used in Table I was applied, and 34% of the specific TSH binding activity when an alternative procedure (17) was used.

The binding of ^{125}I-TSH by the supernatant containing the glycoprotein receptor component or by the aganglioside membrane residue was significantly less sensitive to inhibition by NaCl than was the binding of ^{125}I-TSH to the intact membrane (Fig. 2A). In contrast, the interaction of TSH with gangliosides as monitored by tyrosine fluorescence spectroscopy was highly salt-sensitive (Fig. 2B). The effect of NaCl on the ganglioside-induced fluorescence changes was independent of the specific ganglioside tested and was observed whether the ganglioside was a poor or a good inhibitor of TSH binding to thyroid membranes (3). In each case, salt reduced the fluorescence change caused by that ganglioside. Although these effects of salt on fluorescence changes do not necessarily indicate a reduction in the ganglioside-TSH interaction, the close correlation between the sensitivity of fluorescence changes to salt and the salt sensitivity of ^{125}I-TSH binding to intact membranes (Fig. 2, insert) would suggest this possibility. This interpretation is supported by the recent demonstration that ^{125}I-TSH binding to ganglioside-containing liposomes is similarly salt-sensitive (19).

DISCUSSION

The data presented herein show that solubilization of thyroid plasma membranes by lithium diiodosalicylate is an effective way to segregate the two components of the membrane known to interact with TSH. Thus, the glycoprotein components of the membrane with specific TSH binding activity are released into the supernatant, whereas the gangliosides of the thyroid plasma membrane remain predominantly (85%) in the pellet. The decreased binding activity of the delipidated membrane residue (aganglioside residue) by comparison to the lithium diiodosalicylate supernatant could simply reflect denaturation of the glycoprotein component of the receptor or, since chloroform-methanol solutions are known to extract glycoproteins (20), its extraction. Alternatively, these data raise the possibility that maximum binding by the glycoprotein receptor component might require the presence of some gangliosides or perhaps even of a particular ganglioside not yet defined. The data also do not exclude the possibility that

the remaining TSH binding activity in the ganglioside-rich pellet represents the presence of some residual glycoprotein or some other membrane component.

Nevertheless, the possibility raised by these results is that the ^{125}I-TSH binding activity of the intact plasma membrane is contributed to by both the glycoprotein and the ganglioside components of the membrane. Thus, the salt sensitivity of the intact plasma membrane receptor as well as its high degree of hormonal specificity in regard to other glycoprotein hormones (luteinizing hormone and human chorionic gonadotropin, for example) appears to reflect the ganglioside-TSH interaction. The conclusion concerning the salt sensitivity of the TSH-ganglioside interaction is readily seen in Fig. 2 and has been confirmed in direct ^{125}I-TSH binding experiments using ganglioside-containing liposomes (19). The high degree of hormonal specificity is evident in the close correlation of inhibition of binding with the fluorescence changes effected in the different hormones by the different gangliosides (3, 8, 9) and is in contrast to the increased binding of luteinizing hormone and human chorionic gonadotropin exhibited by the glycoprotein component of the receptor (1, 2). The high affinity of the intact membrane receptor seems to reflect the higher affinity constants exhibited by the glycoprotein receptor fragment (1, 2, 19).

In sum, the data herein, coupled with our previous observations concerning (i) the protease sensitivity of TSH binding and adenylate cyclase stimulation in cultured thyroid cells (21) and (ii) the loss of TSH binding and adenylate cyclase stimulation in a thyroid tumor whose membranes are deficient in their ganglioside content (6), suggest that each component contributes to the function of the intact plasma membrane receptor and that the functional transmission of the TSH message to the thyroid cell machinery requires the presence of both a glycoprotein and glycolipid component in the TSH receptor structure.

The existence of both glycolipid and glycoprotein membrane components with receptor specificity has recently been noted in studies of cryoglobulin interactions with cell membranes (22). It is also evident in the fact that there are both glycolipid and glycoprotein components of the cell with blood group speci-

ficity (23). The latter precedent is especially pertinent since it suggests that the glycolipid and glycoprotein components of the TSH receptor may contain analogous oligosaccharide structures.

It is not clear how the two components function in the binding of TSH and in the transmission of the TSH message to the cell machinery. The binding of TSH may involve both components simultaneously or there may be a sequential interaction with one and subsequent transfer or association with the second, i.e., one can be the primary interaction site or obligatory discriminator and the other can be the coupler to other cell processes. It is hoped that current experiments using liposomes (19) and involving reconstitution of the different components will contribute to the resolution of these questions.

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