ABNORMAL ADENYLATE CYCLASE ACTIVITY AND ALTERED MEMBRANE GANGLIOSIDES IN THYROID CELLS FROM PATIENTS WITH GRAVES' DISEASE

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Received May 16,1977

SUMMARY: Cultured thyroid cells from patients with Graves' disease have elevated cyclic AMP levels when grown in the presence of thyrotropin, and lowered levels in the absence of thyrotropin when compared to thyroid cells from normal individuals or patients with adenomas or colloidonodular goiters. This difference is correlated with an abnormal ganglioside pattern in the membranes from Graves' disease thyroids. These membranes are, however, indistinguishable in their binding of ^{125}I-thyrotropin. These findings are discussed as they relate to current views concerning the structural components of the thyrotropin receptor and the role of these components in the transmission of the thyrotropin message to the cell.

Recent evidence suggests that the mechanism of action of TSH* involves a multistep sequence of events whose major features are, in turn (1-13): interaction with a receptor complex which has both a glycoprotein and a ganglioside component; a conformational change in the TSH molecule; the translocation into the cell membrane of a subunit which has a sequence homology with the cholera toxin A_1 protein and the nonapeptide hormones, oxytocin and vasopressin; a change in state or conformation of the membrane; a change in the electrochemical gradient across the cell membrane; a hormone-responsive adenylate cyclase system; and a cell machinery which is responsive to alterations in cyclic AMP levels.

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* Abbreviations: TSH, thyrotropin; G_{M3}, N-acetylneuraminylgalactosylglucosylceramide; 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

A major question implicit in this schema concerns the role of each receptor component (11), the glycoprotein which has specific TSH binding activity, and the ganglioside, in propagating this sequence of events and in coupling the intramembrane events to changes in adenylate cyclase activity. In this report we describe an abnormality in adenylate cyclase activity and an alteration in membrane gangliosides, using thyroid cells from patients with Graves' disease. We discuss the implication of this correlation to the role of each of the receptor components in the message transmission process, as well as the implications of these observations to the pathogenesis of Graves' disease.

MATERIALS AND METHODS

Human thyroid cells were obtained by trypsinization (14) and were cultured according to a technique previously described (14).† Where appropriate, TSH was added to the medium culture at a concentration of 500 mU/ml.

Patients with colloidonodular goiters were euthyroid but had large thyroids producing compressive phenomena. Toxic adenoma patients were clinically hyperthyroid; their thyroid scans revealed well-defined areas (discrete nodules) with high iodine uptake. Patients with Graves' disease had severe symptoms of hyperthyroidism, large goiters, malignant exophthalmos, high values of thyroid hormone in their sera, and high iodine uptake and release. The scans of their thyroid gland showed an intense and diffuse fixation of radioiodine in an enlarged thyroid gland. The patient with thyroiditis was euthyroid, had a large goiter, and had high serum levels of antithyroglobulin and antimicrosomal antibodies.

Cyclic AMP levels in cells were measured as previously described (14), as was ^{125}I-TSH binding to the membranes of these cells (2, 15, 16). Unless stated otherwise, binding assays contained the following in a total volume of 100 µl: 0.02 M Tris-acetate at pH 6.0, 0.6% bovine serum albumin, and approximately 65,000 cpm of ^{125}I-TSH (2.5 nM), in addition to the membranes. To insure that ^{125}I-TSH binding was specific, control incubations contained either no membranes or a 3,000-fold excess of unlabeled TSH. Binding data are expressed as specific binding, i.e., with control values subtracted. Purified preparations of bovine TSH and ^{125}I-TSH were obtained as previously described (15, 17, 18). Membrane preparations from the various human thyroids were obtained using techniques detailed for bovine thyroid membrane preparations, with the exception that centrifugation of the crude membrane preparations was at 7,000-10,000 x g rather than 3,000 x g (15, 16). Protein was determined by a colorimetric procedure (19) using crystalline albumin as a reference.

Gangliosides were extracted by the following modification of the methods of Yu and Ledeen (20). After washed packed membranes (5-10 mg membrane protein) were extracted once with 20 volumes of chloroform : methanol (1 : 1, v/v) and twice with 10 volumes of chloroform : methanol (2 : 1, v/v), the combined extracts were evaporated to dryness under N_2 and fractionated on 2.5 g of DEAE-Sephadex A-25 (Pharmacia Fine Chemicals, Inc.). The ganglioside fraction was saponified, pelleted by centrifugation, washed 2 times with chloroform : methanol : H_2O (60 : 30 : 4.5, v/v/v) and desalted on 2 g of Sephadex G-25

† We are indebted to Mrs. Ch. Timon and Miss L. Demol for the skillful technical assistance.

superfine (20, 21). Resorcinol spray reagents were used to detect individual gangliosides (22) after the ganglioside fractions were subjected to analytical thin-layer chromatography (2, 21). Standards for these analyses were bovine brain gangliosides obtained as previously described.

RESULTS

Human thyroid cells can be cultured after trypsin treatment of minced human thyroid glands. The optimum trypsin concentration and duration of trypsin treatment was the same as that used in studies of cultured dog thyroid cells (14) independent of the disease state from which the human thyroid cells were obtained. In each case, the human thyroid cells formed a monolayer structure in the absence of TSH in the culture medium. When cultured in the presence of TSH, human thyroid cells from patients with Graves' disease, colloidonodular goiter, toxic adenoma, or thyroiditis, did not form follicle-like structures typical of those seen in the dog system (14) studied under analogous conditions, but did take up iodide and incorporate iodide into thyroglobulin (14, 23). Colloid droplets, analogous to those previously described in dog thyroid cells (23), could be readily identified inside and outside of the cells in each case.

Cyclic AMP levels of the normal human thyroid cells and of cells derived from a colloidonodular goiter, a microfollicular adenoma, a trabecular adenoma, and a toxic adenoma, were similar to cyclic AMP levels of normal dog thyroid cells when measured in their monolayer state, i.e., when cultured in the absence of TSH (Table I). In the presence of TSH, there was an approximately 2-fold increase in cyclic AMP values in all these cells (Table I).

These results were in contrast to the cyclic AMP levels in the thyroid cells from several patients with Graves' disease and one patient with thyroiditis. Cyclic AMP levels in the monolayer cultures of the Graves' disease cells were slightly lower than in monolayer cells derived from the other cell types (Table 1). More important, there was a higher level of cyclic AMP in the Graves' disease cells grown in the presence of TSH. Thus, whereas the usual difference between monolayer and TSH-treated cells was a 2-fold difference in cyclic AMP levels, in the Graves' cells this difference was 4- to 6-fold (Table I).

TABLE I. Cyclic AMP content of human thyroid cell cultures

Origin of thyroid cells	Cyclic AMP (pmoles/mg of protein) [a]	
	Control cells	TSH-treated cells
Normal human thyroid	7,200 ± 900	16,200 ± 1,900
Colloidonodular goiter	6,400 ± 878	14,850 ± 2,935
Microfollicular adenoma	8,200 ± 1,050	17,250 ± 3,500
Trabecular adenoma	7,100 ± 975	16,245 ± 2,845
Toxic adenoma	6,896 ± 2,213	14,800 ± 3,078
Graves' disease		
Patient 1	4,490 ± 950	21,200 ± 2,500
Patient 2	3,650 ± 1,000	23,600 ± 1,700
Patient 3	5,300 ± 700	31,500 ± 1,400
Thyroiditis	4,000 ± 800	24,800 ± 2,400

[a] Average value of 6 different cultures ± 1 S.D. In each case, cell density was within ± 5% at the time of assay.

^{125}I-TSH binding to thyroid plasma membranes from patients with Graves' disease could not be distinguished from ^{125}I-TSH binding to normal human thyroid membranes or to membranes derived from colloidonodular goiters and a toxic adenoma when evaluated as a function of membrane protein concentration (Fig. 1A) or salt concentration (Fig. 1B). Similar results were obtained when ^{125}I-TSH binding was evaluated as a function of time, temperature, and pH (data not shown). The specificity of binding was the same in each case, i.e., ^{125}I-TSH binding was inhibited 98% by unlabeled TSH (5×10^{-6} M) but not at all by this concentration or a 10-fold higher concentration of insulin, glucagon, prolactin, growth hormone, or parathyroid hormone.

Although the total ganglioside content of Graves' disease thyroid membranes appeared to be similar to the ganglioside content of normal human thyroid membranes and membranes from a toxic adenoma, qualitative differences were readily detected on thin-layer chromatograms of the extracted gangliosides (Fig. 2).

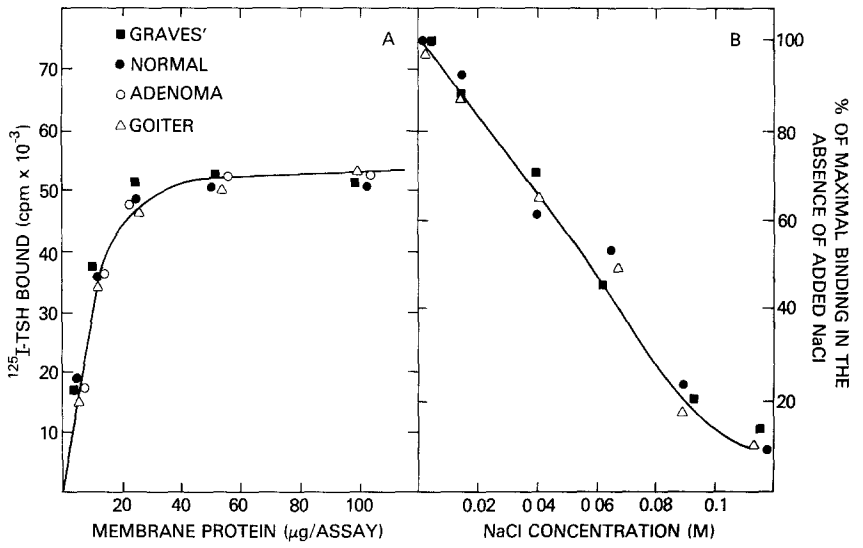

FIG. 1. Binding of ^{125}I-TSH to human thyroid membranes as a function of the amount of membrane protein (A) or the concentration of NaCl (B) in the assay. Assay conditions are detailed in "Materials and Methods."

DISCUSSION

The current report demonstrates that thyroid cells from patients with Graves' disease have an abnormally sensitive adenylate cyclase system by comparison to normal human thyroid cells or thyroid cells derived from the glands of patients with several other thyroid disease states. The abnormal cyclic AMP response to TSH has not been correlated with any abnormality in ^{125}I-TSH binding by these membranes but has been shown to be associated with an abnormality in membrane gangliosides, i.e., with one of the membrane components identified with TSH receptor function (2-13).

In regard to the ganglioside abnormalities, it is clear there are decreases in some gangliosides and increases in others. Since in a recent study (24), we have shown that a minor ganglioside component, i.e., one which is 0.015% of the total ganglioside content of the bovine thyroid, has the highest ability to inhibit TSH binding, and since we know little about the structural determinants in the oligosaccharide moiety of human thyroid gangliosides likely to accentuate

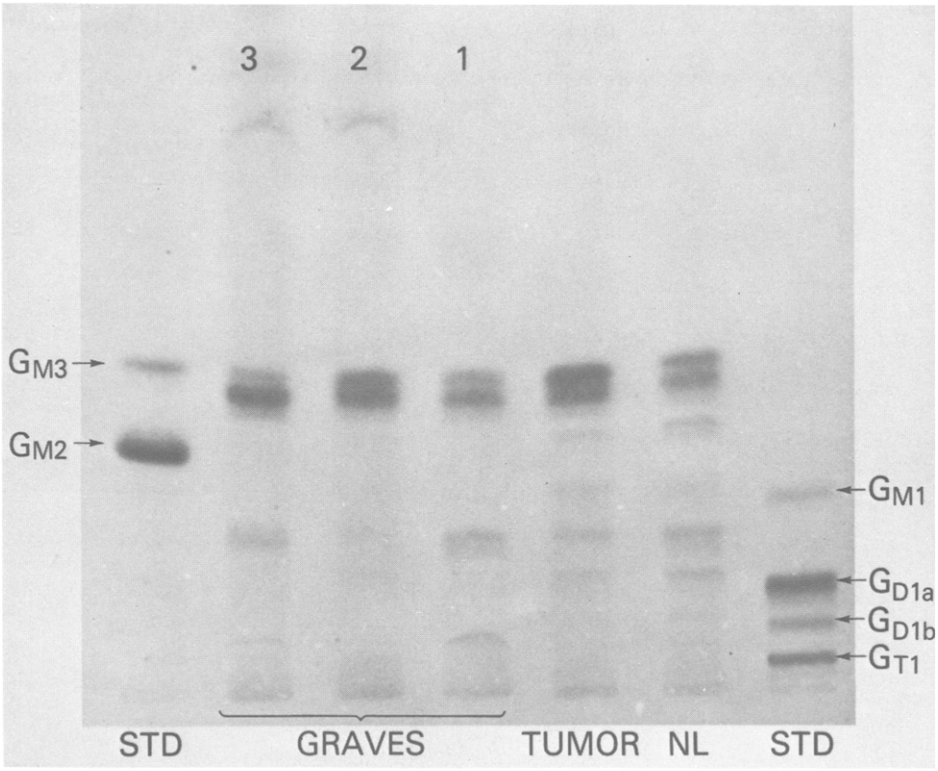

FIG. 2. Thin-layer chromatography of gangliosides isolated from the thyroid membranes of a normal human, from a patient with a toxic adenoma, and from 3 patients with Graves' disease. Lipid-bound sialic acid recovered from each membrane preparation was the same per mg membrane protein; equal amounts of total lipid-bound sialic acid were applied to each lane. The thyroid membranes used in these experiments were all derived from the glands of female patients.

or decrease the interaction with TSH, the change of any individual ganglioside component cannot at this time be rigorously interpreted in terms of its interaction with TSH. Nevertheless, the data are suggestive that the ganglioside components of the receptor may be more directly associated with coupling of the hormone message to the cyclase response rather than with the initial hormonal interaction with the receptor.

In regard to the implication of these results to the pathogenesis of Graves' disease, several comments should be made. Abnormalities in glycolipids may well express themselves as alterations in oligosaccharide determinants on the surface of the cell. The relationship of such abnormalities to the development or the

action of autoantibodies in Graves' disease, especially microsomal autoantibodies, is unknown, but may be a fertile area of exploration. This relationship is especially pertinent in the light of current evidence that immunoglobulins in the sera of patients with Graves' disease can interact with the thyroid membrane and that this interaction is etiologically related to thyroid dysfunction (25-30). Experiments concerning the effect of antibodies on the ganglioside composition of these membranes are currently being actively pursued, as are experiments concerned with the interaction of autoantibodies in Graves' sera with individual ganglioside components.

In conclusion, it appears that studies using human thyroid cells in culture which are derived from different disease states will offer additional insights into the pathophysiological effects and etiology of these disease states and will contribute to our knowledge of the coupling between different structural components of the TSH receptor and the adenylate cyclase system.

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