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Increased Expression of the Na^+/I^- Symporter in Cultured Human Thyroid Cells Exposed to Thyrotropin and in Graves' Thyroid Tissue*

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ABSTRACT

The Na^+/I^- symporter (NIS) is important in hormone synthesis in the thyroid gland. NIS activity, as reflected by I^- uptake, was increased by TSH (1 mU/mL) or forskolin (10 μmol/L) in primary cultured human thyroid cells. Northern blot analysis revealed that incubation of these cells with TSH or forskolin for 24 h increased the abundance of NIS messenger ribonucleic acid (mRNA) 2.3- and 2.5-fold, respectively. Immunoblot analysis revealed 2.7- and 2.4-fold increases, respectively, in the amount of NIS protein after 48 h, suggesting that elevated levels of intracellular cAMP induced the expression of NIS in human thyrocytes. We then studied the levels of NIS mRNA and protein in Graves' thyroid tissue and found that the amount of NIS mRNA in thyroid tissue from individuals with Graves' disease (n = 5) was 3.8 times that in normal thyroid tissue (n = 5). The abundance of NIS mRNA was significantly correlated with that of thyroid peroxidase or thyroglobulin mRNAs, but not with that of TSH receptor mRNA, in the Graves' and normal thyroid tissue specimens. The amount of NIS protein was also increased 3.1-fold in Graves' thyroid tissue compared with that in normal thyroid tissue. The increased expression of NIS may thus contribute to the development of Graves' disease. (*J Clin Endocrinol Metab* **82:** 3331–3336, 1997)

THE TRANSPORT of iodide (I^-) into thyroid cells is required for the synthesis of thyroid hormone (1). Intracellular I^- is subsequently incorporated into thyroglobulin (Tg) in a reaction that is catalyzed by thyroid peroxidase (TPO). Both this reaction and I^- transport are stimulated by TSH. The Na^+/I^- symporter (NIS) plays an important role in I^- transport into the thyroid follicular cells. However, the molecular mechanism in the regulation of NIS by TSH remained unclear because neither the gene nor the protein of NIS had been identified. On the other hand, it is known that I^- uptake activity is increased in patients with Graves' disease (2) and that patients with congenital goiter caused by hypothyroidism reportedly exhibit a partial defect of I^- transport (3–5). Thus, NIS seems to be implicated in the pathogenesis and development of these diseases.

Dai *et al.* (6) have recently cloned and characterized the complementary DNA (cDNA) for rat NIS. Subsequently, using recombinant rat NIS protein, we reported that some patients with autoimmune thyroid disease, such as Hashimoto's thyroiditis and Graves' disease, might possess an autoantibody against NIS and that the IgGs from patients with Hashimoto's thyroiditis could affect NIS activity reflected by I^- uptake (7, 8).

We have now cloned and sequenced the human NIS cDNA that contains the entire coding sequence and examined the effect of TSH on NIS messenger ribonucleic acid (mRNA) and protein in cultured human thyroid cells as well as measured NIS mRNA and protein in normal and Graves' thyroid tissues in the present study.

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* The nucleotide sequence of human sodium iodide symporter reported in this paper has been submitted to the GenBank database. The accession number assigned to this sequence is D87920.

Materials and Methods

Cell cultures

Thyroid tissue was obtained from a patient with Graves' disease who underwent a subtotal thyroidectomy. The diagnosis of Graves' disease was based on conventional criteria (diffuse goiter, hyperthyroidism, and exophthalmos); TSH binding inhibitor immunoglobulin (TBII) activity was 26% before surgery. Protocols were approved by the institutional ethics board of Yamanashi Medical University. Thyroid cells were prepared and cultured as described previously (9–12). In brief, thyroid tissue was digested with collagenase type II (1 mg/mL; Sigma Chemical Co., St. Louis, MO) and dispase II (5 mg/mL; Boehringer Mannheim, Mannheim, Germany). After being centrifuged, resuspended, and washed three times, follicles were seeded in 24-well culture plates or 10-cm culture dishes and cultured in Coon's modified Ham's F-12 medium (Sigma) containing insulin (10 μg/mL), transferrin (5 μg/mL), somatostatin (10 ng/mL), hydrocortisone (10 nmol/L), glycyl-L-histidyl-L-lysine (10 ng/mL), TSH (5 mU/mL; Sigma), penicillin (100 U/mL), streptomycin (100 μg/mL), and amphotericin B (2.5 μg/mL). If indicated, 1% calf serum (Life Technologies, Grand Island, NY) was also added to the medium. The cells were incubated for 2 days and then maintained for 5 days in the same medium lacking TSH (5H medium). Culture medium was replaced every other day. The cells were then incubated for the indicated times in 5H medium containing TSH (1 mU/mL), forskolin (10 μmol/L), mercaptomethylimidazol (MMI; 1 mmol/L), or TSH (1 mU/mL) plus MMI (1 mmol/L) before measurement of I^- uptake or NIS mRNA or protein.

Iodide uptake

Cells in 24-well plates were rinsed with I^- uptake buffer [137 mmol/L NaCl, 5.4 mmol/L $MgCl_2$, 1.3 mmol/L $CaCl_2$, 0.4 mmol/L $MgSO_4$, 0.5 mmol/L $MgCl_2$, 0.4 mmol/L Na_2HPO_4, 0.44 mmol/L KH_2PO_4, 5.55

mmol/L glucose, and 10 mmol/L HEPES (pH 7.3)] and then incubated for 2 h at 37 C with 100 nmol/L $^{125}I^-$ (50 mCi/mmol) in the same buffer (9, 13). The cells were rapidly rinsed and scraped from each well, and the associated radioactivity was measured with a γ-counter. The radioactivity was normalized to the cellular protein content measured in the same cells. For the assay of I^- uptake in CHO-K1 cells transfected with human NIS cDNA in pcDNA3 (Invitrogen, San Diego, CA), the cells were incubated with the same I^- uptake buffer containing $^{125}I^-$ for the indicated times as described previously (7).

Cloning and sequencing of human NIS cDNA

We screened 1 million independent recombinant bacteriophage from a human thyroid follicular carcinoma cDNA library in λgt11 (HL1009b, Clontech, Palo Alto, CA) with a rat NIS cDNA probe as described previously (7). Inserts from purified recombinant bacteriophage obtained from four rounds of library screening (14) were excised with *Eco*RI (Takara, Ohtsu, Japan) and subcloned into pBluescript SK^+ (Stratagene, La Jolla, CA). Cloned inserts were subcloned into the M13 mp18 phage vector and sequenced.

Northern blot analysis

Total RNA (15 μg) from cultured cells or from specimens of five normal and five Graves' human thyroids (obtained from patients undergoing hemithyroidectomy for benign adenoma or papillary cancer, or subtotal thyroidectomy for Graves' disease; TBII activities in Graves' patients ranged from 56.0–66.3%) was separated on a 1% agarose gel containing formaldehyde and transferred to a nylon filter as described previously (15). A human NIS RNA probe was prepared by the *in vitro* transcription of pcDNA3 containing the human NIS cDNA with SP6 RNA polymerase (Pharmacia LKB, Tokyo, Japan). Other probes were prepared by labeling cDNAs corresponding to rat β-actin, human TPO, rat Tg, and human TSH receptor (TSHR) as previously described (16). The filters were hybridized with the probes and washed, and mRNA signals were quantitated with a BAS2000 system (Fujix, Tokyo, Japan).

Immunoblot analysis

Membrane fractions were prepared from cultured cells or thyroid tissue in a solution containing 10 mmol/L Tris-HCl (pH 7.4), 5 mmol/L NaCl, 1 mmol/L ethylenediamine-N,N,N′,N′-tetraacetic acid, 1 mmol/L phenylmethylsulfonylfluoride, and leupeptin (50 μg/mL); subjected to electrophoresis on a 10% polyacrylamide gel containing 0.1% SDS; and transferred to a nitrocellulose filter. Immunoblot analysis was performed with antiserum (1:500) generated with glutathione-*S*-transferase/N-terminal portion of rat NIS (amino acids 1–231) fusion protein as described previously (7, 8). The abundance of immunoreactive human NIS was quantitated with a scanning densitometer (model GT-8000, Epson, Tokyo, Japan).

Statistical analysis

Data are presented as the mean ± SE and were analyzed by Student's *t* test or linear regression. When multiple comparisons were made, statistical significance was determined by ANOVA with the Bonferroni/Dunn *post-hoc* test. $P < 0.05$ was considered statistically significant.

Results

Iodide uptake by cultured cells

The uptake of I^- by cultured human thyroid cells was stimulated by the addition of TSH (1 mU/mL) or forskolin (10 μmol/L) in a time-dependent manner (Fig. 1), consistent with previous observations (9, 10, 13). Cells exposed to 1% calf serum during culture showed a reduced response to TSH (data not shown), again as demonstrated previously (9, 10). MMI had no effect on I^- uptake in the absence or presence of TSH.

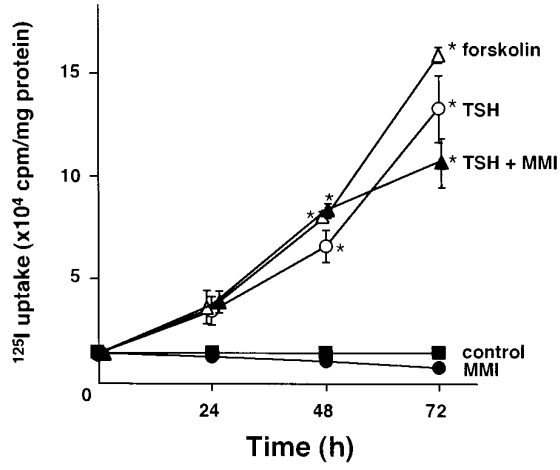

FIG. 1. Uptake of I^- by cultured human thyrocytes. The cells were seeded and cultured without serum. After incubation for 5 days in 5H medium (without TSH), cells were incubated for the indicated times in the same medium containing TSH (1 mU/mL; ○), forskolin (10 μmol/L; △), MMI (1 mmol/L; ●), TSH plus MMI (▲), or no additives (■). I^- uptake was then determined as described in *Materials and Methods*. Data are the mean ± SE of triplicate wells. *, $P < 0.005$ *vs.* control (no additives), by Bonferroni/Dunn *post-hoc* test.

Cloning of human NIS

We were unsuccessful in our attempt to detect human NIS mRNA by Northern blot analysis with rat NIS cDNA as a probe, which thus necessitated the cloning of human NIS cDNA. Screening of a human thyroid follicular carcinoma cDNA library with rat NIS cDNA identified an approximately 1.6-kilobase (kb) insert that contained 240 bp of the 5′-untranslated region and 1333 bp of the open reading frame of human NIS cDNA. We then screened the library with the cloned 1.6-kb DNA fragment as a probe and obtained an approximately 2.4-kb cDNA that contained the entire coding sequence. The 1929-bp open reading frame encodes a protein of 643 amino acids, as previously described (17). Figure 2 shows replacements of nucleotide and deduced amino acids in our findings. Three amino acid substitutions were found: Pro^{102}, Gln^{536}, and Gln^{556} instead of Ala, Thr, and Ser, respectively. Other nucleotide replacements were T (at the position of 307, A in ATG initiation codon is designated 1), G (1338), and G (1548) instead of C, C, and A, respectively, with no amino acid substitutions. CHO-K1 cells transfected with the cloned human NIS cDNA showed marked I^- uptake activity, whereas CHO-K1 cells transfected with the pcDNA3 alone did not have I^- uptake (Fig. 3).

Regulation of human NIS mRNA and protein in cultured cells

As shown in Fig. 1, TSH (1 mU/mL) and forskolin (10 μmol/L) up-regulated I^- uptake activity, so we determined human NIS mRNA levels in the presence of these agents. Northern blot analysis of total RNA from cultured human thyroid cells with a human NIS RNA probe revealed marked hybridization with an approximately 3.5-kb mRNA (Fig. 4A). Incubation of cells for 24 h with TSH or forskolin increased the amount of NIS mRNA 2.3- and 2.4-fold, respectively (Fig. 4). MMI (1 mmol/L) had little effect on NIS mRNA abun-

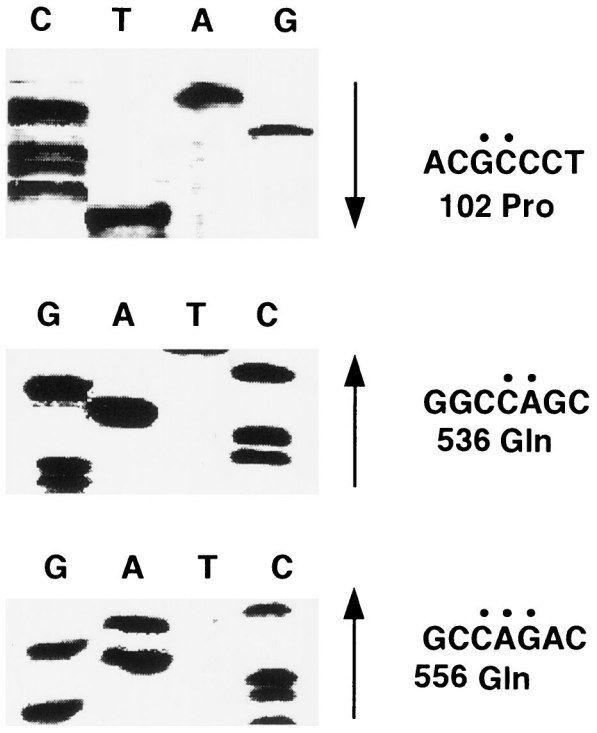

FIG. 2. Sequence analysis and amino acid substitutions. Human NIS cDNA was subcloned to M13 phage and sequenced. Three amino acids (Ala, Thr, and Ser shown in Ref. 17) were replaced by Pro, Gln, and Gln, respectively. *Dots* indicate the nucleotide substitutions.

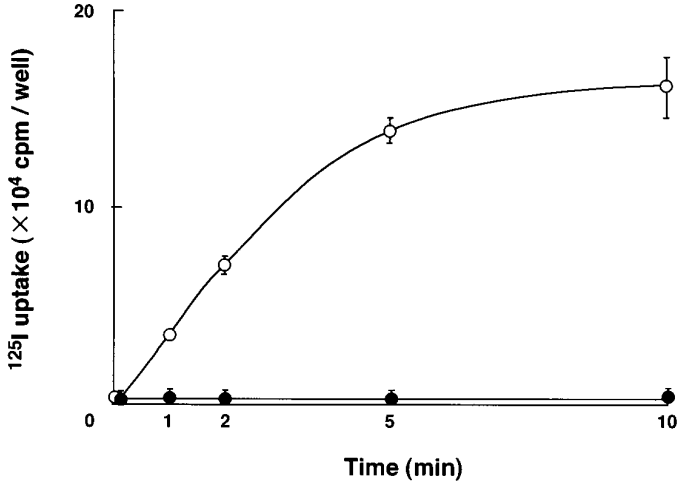

FIG. 3. Uptake of I^- by CHO-K1 cells that stably expressed recombinant human NIS. CHO-K1 cells were transfected with (○) or without (●) human NIS cDNA in pcDNA3, and I^- uptake was assayed after incubation of cells for the indicated times with $^{125}I^-$. Data are the mean ± SE of three separate experiments with different batches of cells on different days.

dance. Immunoblot analysis with antiserum to rat NIS detected an approximately 77-kDa immunoreactive protein in cultured human thyroid cells, consistent with our previous observations (7); weak signals were detected at 88 and 60 kDa, but these were also apparent with serum preadsorbed with rat NIS, whereas 77-kDa protein disappeared (Fig. 4A, lane f). Incubation of cells with TSH or forskolin for 48 h increased the amount of NIS protein 2.7- and 2.4-fold, respectively. MMI had no effect on the abundance of human NIS protein.

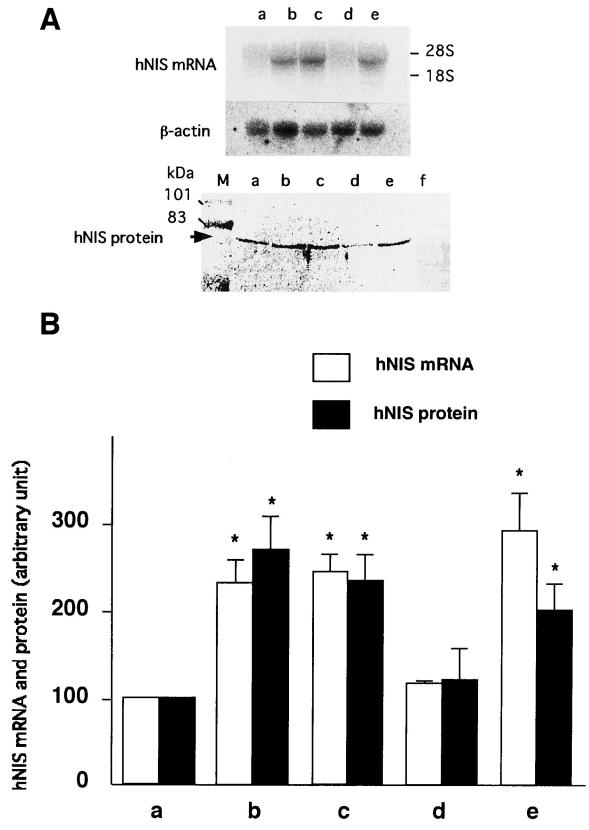

FIG. 4. Regulation of NIS mRNA and protein abundance in cultured human thyroid cells. A, Total RNA from cells incubated for 24 h with various agents was subjected to Northern blot analysis with human NIS RNA probe or rat β-actin cDNA probes (*upper panel*). The positions of 28S and 18S ribosomal RNA are indicated. Membrane fractions prepared from cells incubated for 48 h with various agents were subjected to immunoblot analysis with antiserum (1:500) to rat NIS (*lower panel*, lanes a–e). The positions of 101- and 83-kDa molecular size standards (lane M) are indicated. Lanes correspond to cells incubated in the absence (a and f) or presence of TSH (1 mU/mL; b), forskolin (10 μmol/L; c), MMI (1 mmol/L; d), or TSH plus MMI (e). Immunoblot analysis in lane f was performed with antiserum (1:500) that was preadsorbed with rat NIS. Data represent typical experiments that were obtained three individual dishes. B, Quantitative analysis of data from three individual dishes corresponding to that shown in A. Data are the mean ± SE and are expressed relative to the control values (lane a). *, $P < 0.05$ *vs.* control with Bonferroni/Dunn *post-hoc* test.

Northern blot analysis of human NIS in normal and Graves' thyroid tissues

As human NIS was up-regulated by TSH and forskolin in these experiments, it was expected that the expression of human NIS was increased in Graves' thyroid compared with that in normal thyroid. Northern blot analysis revealed the amounts of NIS mRNA in thyroid tissue from five patients with Graves' disease to be markedly greater than those in five specimens of normal thyroid tissue (Fig. 5A). The abundance of both TPO and Tg mRNAs, but not that of TSHR mRNA, also appeared increased in Graves' thyroid tissue. Quantitative densitometry and normalization of the amounts of

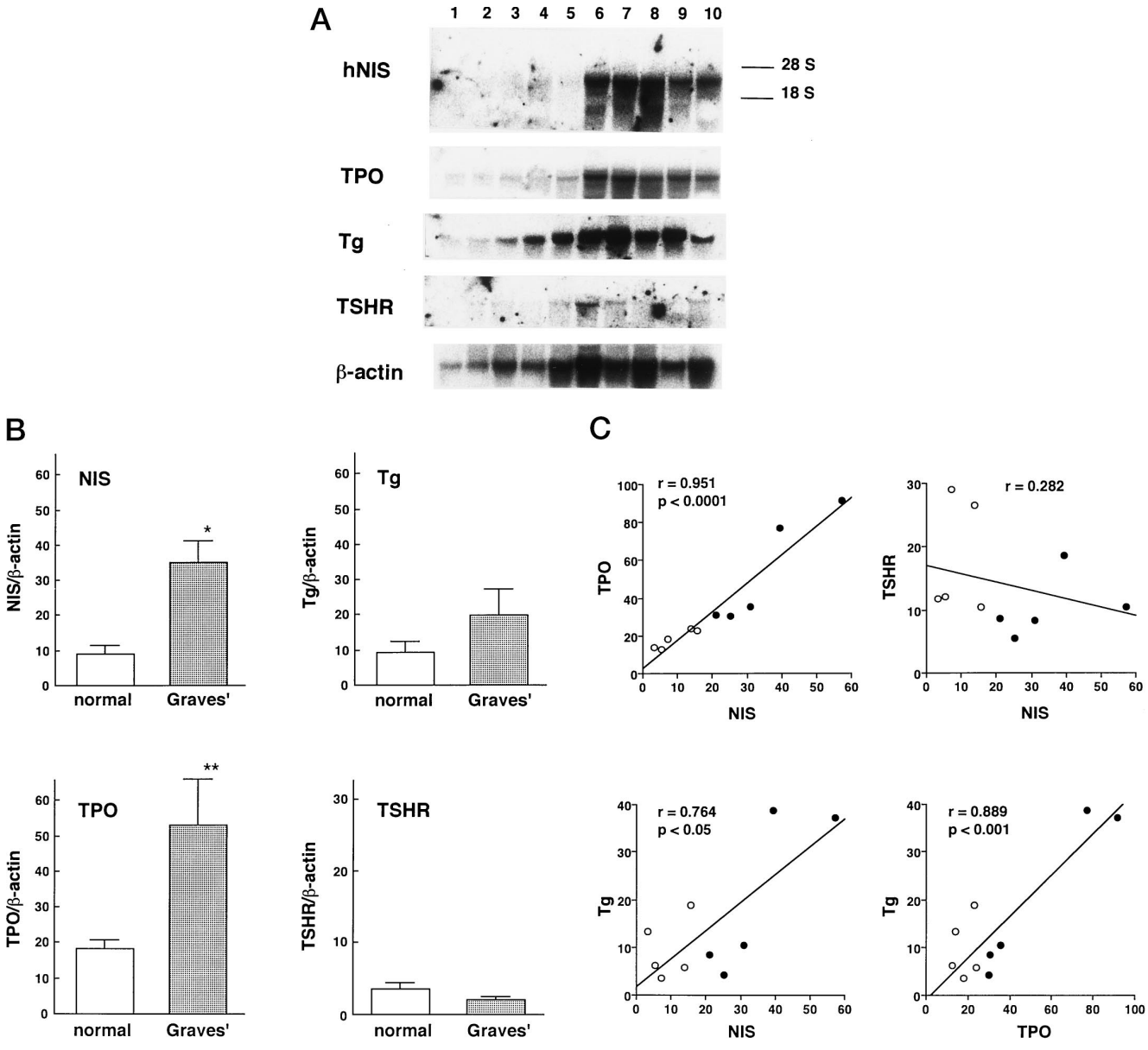

FIG. 5. Northern blot analysis of NIS, TPO, Tg, and TSHR mRNA in normal and Graves' thyroid tissue. A, Total RNA from specimens of normal (lanes 1–5) and Graves' (lanes 6–10) thyroid tissue was subjected to Northern analysis with probes specific for NIS, TPO, Tg, TSHR, or β-actin mRNA as indicated. B, The amounts of NIS, TPO, Tg, and TSHR mRNAs were quantitated by densitometry and expressed relative to the amount of β-actin mRNA. Data are the mean ± SE ($n = 5$). *, $P < 0.01$; **, $P < 0.05$ (*vs.* normal, by Student's t test). C, Correlation analysis of the relations among the normalized amounts of NIS, TPO, Tg, and TSHR mRNAs in normal (○) and Graves' (●) thyroid specimens.

NIS, TPO, Tg, and TSHR mRNAs relative to the amount of β-actin mRNA revealed significant increases of approximately 3.8- and 2.9-fold in NIS and TPO mRNAs, respectively, in Graves' thyroid tissue (Fig. 5B). Then we studied the correlation between the expression levels of these thyroid-specific genes. The amount of NIS mRNA in all tissue specimens was significantly correlated with those of TPO and Tg mRNAs (Fig. 5C); there was also a significant correlation between the amounts of TPO and Tg mRNAs, but not between the amount of TSHR mRNA and that of NIS, TPO, or Tg mRNA (r = 0.282, r = 0.107, and r = 0.104, respectively).

Immunoblot analysis of NIS in normal and Graves' thyroid tissue

Immunoblot analysis of membrane fractions prepared from three normal and four Graves' thyroid specimens was performed with antiserum to rat NIS. The amount of NIS protein in Graves' thyroid specimens was 3.1 times that in normal thyroid tissue (Fig. 6).

Discussion

TSH/cAMP has previously been shown to stimulate I^- uptake in both the continuous rat thyroid cell line FRTL-5 and primary cultures of human thyroid cells (9, 10, 13). Our

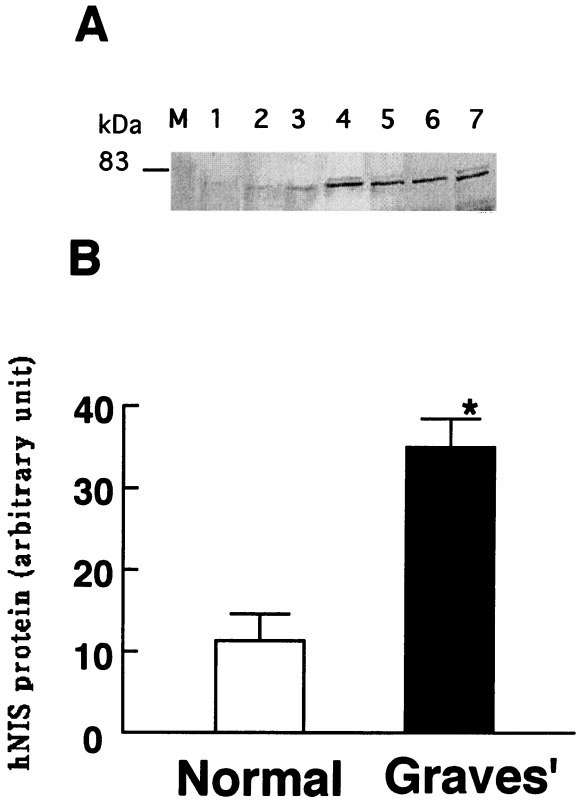

FIG. 6. Immunoblot analysis of NIS protein in normal and Graves' thyroid tissue. A, Membrane fractions prepared from specimens of normal (lanes 1–3) and Graves' (lanes 4–7) thyroid tissue were subjected to immunoblot analysis with antiserum (1:500) to rat NIS. Lane M, Molecular size markers. B, Quantitative analysis of the data in A. Values are the mean ± SE of three normal and four Graves' thyroid specimens. *, $P < 0.005$ *vs.* normal thyroid, by Student's *t* test.

demonstration that TSH and forskolin increased I^- uptake by cultured human thyroid cells is thus consistent with these previous observations. Serum-free cultures of human cells responded well to TSH and forskolin in a time-dependent manner. The presence of 1% serum reduced I^- uptake stimulated by TSH as described previously (9, 10). These data suggest that our cultures followed the previous experiments (9, 10) and were thought to be valid. MMI, known to be a goitrogen *in vivo*, had no effect on I^- uptake directly in the primary cultured cells. Incubation for I^- uptake was usually performed for 40–120 min in primary cultured human thyroid cells, as described previously (9, 10, 18, 19). Two-hour incubation for I^- steady state uptake could reflect not only I^- transport, but also other factors, such as backflux rate by nonspecific leak, cell water content, and protein binding; however, the major part of the uptake activity could reflect I^- transport stimulated by TSH/forskolin. Therefore, we next determined NIS expression, which may be involved in the increase in I^- uptake.

We also cloned human NIS cDNA and detected three differences in the deduced amino acid sequence from that determined by Smanik *et al.* (17). These differences in amino acids might not appear to affect I^- uptake activity, given the marked activity in cells transfected with our cloned entire NIS cDNA, and may possibly represent polymorphisms.

TSH and forskolin up-regulate NIS mRNA and protein in FRTL-5 cells, actions that may underlie at least in part the effect of the agents on I^- uptake activity (20); NIS mRNA was markedly increased after 24 h and NIS protein after 48 h of incubation with TSH. We have now shown that TSH and forskolin markedly increased the amounts of NIS mRNA and protein in primary cultured human thyroid cells at these same time points. Thus, the TSH/cAMP pathway appears to up-regulate NIS gene expression and NIS protein abundance, resulting in an increase in I^- uptake activity in human thyroid cells.

In individuals with Graves' disease, an up-regulation of thyroid function, as reflected in increased uptake of I^- (2) and increased hormone production as a result of increased H_2O_2 generation and synthesis of TPO and Tg (21–23), appears to contribute to both the progression and duration of the disease. We have now shown that the amounts of NIS mRNA and protein are increased in thyroid tissue from Graves' patients. The significant correlation between the abundance of NIS mRNA and that of TPO or Tg mRNAs in normal and Graves' thyroid tissue suggests that NIS may be a marker of thyroid cell differentiation. We have previously shown that the amounts of Tg and TSHR mRNAs are reduced in neoplastic thyroid tissue relative to those in normal thyroid, suggesting that the abundance of these mRNAs reflects the extent of tissue differentiation (16). Therefore, NIS mRNA expression should be determined not only in Graves' thyroid but also in benign and malignant neoplasms. The correlation between NIS and TPO or Tg mRNAs also suggests that the mechanism of NIS gene expression might be similar to that of the TPO and Tg genes. The expression of TPO and Tg genes is stimulated by a cAMP pathway in primary cultures of human thyroid cells (24, 25). Given the marked TBII activity detected in all of the Graves' patients in the present study, it is possible that the increased NIS mRNA and protein concentrations in the corresponding thyroid tissue were attributable to increased cAMP accumulation induced by TBII. Increased expression of both NIS and TPO may contribute to the development of Graves' disease.

Investigation of the regulation of human NIS gene expression provides an important information that will be useful in defining the precise role of this protein and may, therefore, produce a practical approach to elucidate thyroid disease.

References

1. **Taurog A.** 1986 Hormone synthesis: thyroid iodine metabolism. In: Ingbar SH, Braverman LE, eds. Werner's the thyroid. Philadelphia: Lippincott; 53–97.
2. **Cavalieri RR.** 1986 Quantitive *in vivo* tests. In: Ingbar SH, Braverman LE, eds. Werner's the thyroid. Philadelphia: Lippincott; 445–458.
3. **Toyoshima K, Matsumoto Y, Nishida S, Yabuuchi H.** 1977 Five cases of absence of iodide concentrating mechanism. Acta Endocrinol (Copenh). 84:527–537.
4. **Saito K, Yamamoto K, Nishida M, et al.** 1981 Goitrous hypothyroidism due to iodide-trapping defect. J Clin Endocrinol Metab. 53:1267–1272.
5. **Wofff J.** 1983 Congenital goiter with defective iodide transport. Endocr Rev. 4:240–253.
6. **Dai G, Levy O, Carrasco N.** 1996 Cloning and characterization of the thyroid iodide transporter. Nature. 379:458–460.
7. **Endo T, Kogai T, Nakazato M, Saito T, Kaneshige M, Onaya T.** 1996 Autoantibody against Na^+/I^- symporter in the sera of patients with autoimmune thyroid disease. Biochem Biophys Res Commun. 224:92–95.
8. **Endo T, Kaneshige M, Nakazato M, Kogai T, Saito T, Onaya T.** 1996 Autoantibody against thyroid iodide transporter in sera from patients with Hashimoto's thyroiditis possesses iodide transport inhibitory activity. Biochem Biophys Res Commun. 228:199–202.

9. **Roger P, Taton M, Van Sande J, Dumont JE.** 1988 Mitogenic effects of thyrotropin and adenosine 3′,5′-monophosphate in differentiated normal human thyroid cells *in vitro*. J Clin Endocrinol Metab. 66:1158–1165.
10. **Kraiem Z, Sadeh O, Yosef M.** 1991 Iodide uptake and organification, triiodothyronine secretion, cyclic AMP accumulation and cell proliferation in an optimized system of human thyroid follicles cultured in collagen gel suspended in serum-free medium. J Endocrinol. 131:499–506.
11. **Saito T, Lamy F, Roger P, Lecocq R, Dumont JE.** 1994 Characterization and identification as cofilin and destrin of two thyrotropin- and phorbol ester-regulated phosphoproteins in thyroid cells. Exp Cell Res. 212:49–61.
12. **Rasmussen AK, Kayser L, Perrild H, Brandt M, Bech K, Feldt-Rasmussen U.** 1996 Human thyroid epithelial cells cultured in monolayers. I. Decreased thyroglobulin and cAMP response to TSH in 12-week-old secondary and tertiary cultures. Mol Cell Endocrinol. 116:165–172.
13. **Weiss SJ, Philp NJ, Ambesi-Impiombato FS, Grollman EF.** 1984 Thyrotropin-stimulated iodide transport mediated by adenosine 3′,5′-monophosphate and dependent on protein synthesis. Endocrinology. 114:1099–1107.
14. **Ikeda M, Saito T, Endo T, Tsurugi K, Onaya T.** 1991 Thyrotropin stimulates the expression of an acidic ribosomal protein, P0, messenger ribonucleic acid in cultured rat thyroid (FRTL) cells. Endocrinology. 128:2540–2547.
15. **Sambrook J, Fritsch EF, Maniatis T.** 1989 Molecular cloning: A laboratory manual, 2nd ed. Cold Spring Harbor: Cold Spring Harbor Laboratory.
16. **Ohta K, Endo T, Onaya T.** 1991 The mRNA levels of thyrotropin receptor, thyroglobulin and thyroid peroxidase in neoplastic human thyroid tissues. Biochem Biophys Res Commun. 174:1148–1153.
17. **Smanik PA, Liu Q, Furminger TL, et al.** 1996 Cloning of the human sodium iodide symporter. Biochem Biophys Res Commun. 226:339–345.
18. **Misaki T, Miyamoto S, Sayeedul A, et al.** 1996 Tumoricidal cytokines enhance radioiodine uptake in cultured thyroid cancer cells. J Nucl Med. 37:646–648.
19. **Mothersill C.** 1995 Human thyroid culture. In: O'Hare S, Atterwill CK, eds. Methods in molecular biology. Totowa: Humana Press; vol 43:25–31.
20. **Kogai T, Endo T, Saito T, Miyazaki A, Kawaguchi A, Onaya T.** 1997 Regulation by thyroid stimulating hormone of sodium/iodide symporter gene expression and protein levels in FRTL-5 cells. Endocrinology. 138:2227–2232.
21. **Gerard C, Lefort A, Libert F, Christophe D, Dumont JE, Vassart G.** 1988 Control of thyroperoxidase and thyroglobulin transcription by cAMP: evidence for distinct regulatory mechanisms. Mol Endocrinol. 3:2110–2118.
22. **Chazenbalk G, Magnusson RP, Rapoport B.** 1987 Thyrotropin stimulation of cultured thyroid cells increases steady state levels of the messenger ribonucleic acid for thyroid peroxidase. Mol Endocrinol. 1:913–917.
23. **Vassart G, Dumont JE.** 1992 The thyrotropin receptor and the regulation of thyrocyte function and growth. Endocr Rev. 13:596–611.
24. **Collison KS, Banga JP, Barnett PS, Huang GC, McGregor AM.** 1991 Autoantibody stimulation of the human thyrotropin receptor: regulation of adenylate cyclase activity, thyroglobulin and thyroid peroxidase mRNA levels in primary cultures of Graves' thyroid tissue. Clin Exp Immunol. 86:61–65.
25. **Collison KS, Banga JP, Barnett PS, Kung AWC, McGregor AM.** 1989 Activation of the thyroid peroxidase gene in human thyroid cells: effect of thyrotrophin, forskolin and phorbol ester. J Mol Endocrinol. 3:1–5.347.

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