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Analysis of Human Sodium Iodide Symporter Gene Expression in Extrathyroidal Tissues and Cloning of Its Complementary Deoxyribonucleic Acids from Salivary Gland, Mammary Gland, and Gastric Mucosa

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ABSTRACT

The ability to concentrate iodide is a fundamental property of normally functioning thyroid tissue and represents the first step in the production of thyroid hormones. Iodide uptake has been demonstrated in various extrathyroidal tissues, including salivary gland, gastric mucosa, and lactating mammary gland. Recently, cloning and molecular characterization of the human sodium iodide symporter (hNIS) have been reported; however, the patterns of hNIS gene expression in human tissues have remained unidentified. To examine the profiles of human hNIS gene expression in various normal human tissues, we performed high-stringency Northern blot analysis using a ^{32}P-labeled hNIS-specific complementary DNA (cDNA) probe (nucleotides 1184–1667). To detect rare hNIS transcripts in small tissue samples, RT-PCR was performed with a pair of hNIS-specific oligonucleotide primers designed to amplify a portion (nucleotides 1184–1667) of the hNIS gene. hNIS-specific transcripts were confirmed by Southern hybridization using a digoxigenin-labeled internal hNIS-specific oligonucleotide probe (nucleotides 1460–1477). To monitor cDNA integrity and quantity, and to rule out DNA contamination and illegitimate transcription, all samples were coamplified with two pairs of intron-spanning primers designed to amplify fragments of the human β-actin and thyroglobulin genes, respectively. Using Northern blot analysis, hNIS transcripts of approximately 4 kb were detected in thyroid gland and parotid gland but not in a broad range of endocrine and nonendocrine tissues. RT-PCR and Southern hybridization revealed hNIS gene expression in thyroid gland, salivary gland, parotid gland, submandibular gland, pituitary gland, pancreas, testis, mammary gland, gastric mucosa, prostate and ovary, adrenal gland, heart, thymus, and lung. By contrast, hNIS transcripts were not detected in normal orbital fibroblasts, colon, and nasopharyngeal mucosa. To further analyze hNIS gene sequences in parotid gland, mammary gland, and gastric mucosa, the EXPAND High Fidelity PCR System and three sets of overlapping NIS oligonucleotide primers were used for amplification and cloning. The resulting PCR products were subcloned into pBluescript-SK II (−) vector, and at least two independent cDNA clones derived from each tissue were subjected to automated sequencing. The nucleotide sequences of hNIS cDNA derived from parotid gland, mammary gland, and gastric mucosa revealed full identity with the recently published human thyroid-derived NIS cDNA sequence. In conclusion, our results demonstrate markedly variable levels of hNIS gene expression in several extrathyroidal tissues. Although the physiological role of hNIS in these tissues awaits further study, our results suggest that the capacity to actively transport iodine may be a feature common to several secretory and endocrine tissues. The diminished capacity to transport and concentrate iodide in extrathyroidal tissues (such as parotid gland, mammary gland, and gastric mucosa), compared with thyroid gland, does not seem to be caused by an altered primary structure of the hNIS cDNA. Variability of NIS gene expression levels in normal extrathyroidal tissues may rather be caused by differences in NIS gene transcriptional activity. Further studies will address this hypothesis and examine the mechanisms of tissue-specific regulation of NIS gene expression. (*J Clin Endocrinol Metab* **83:** 1746–1751, 1998)

THE ABILITY to transport and concentrate iodide within the thyroid gland represents the first step in the production of the iodine-containing thyroid hormones. Iodide uptake occurs across the basolateral membrane of polarized thyroid follicular cells and has been shown to be an active transport driven by an inwardly directed Na^+ gradient (1). Recently, cloning of the sodium iodide symporter (rNIS) from a Fisher rat thyroid line (FRTL-5)-derived complementary DNA (cDNA) library (2, 3, 4), and of its human homolog (hNIS) from a human thyroid cDNA library (5), have been reported. An open reading frame from nucleotides 348-2276 of the hNIS gene encodes a protein of 643 amino acids with a predicted molecular mass of 68.7 kDa. The amino acid sequence encoded by hNIS shows 84% identity to the rNIS (2). The hNIS represents an intrinsic membrane protein with 12 putative transmembrane domains (5). The coding region of hNIS contains 15 exons that are interrupted by 14 introns, and the nucleotide sequences of each exon-intron junction have recently been reported (6).

The thyroid gland shares its capacity to actively accumulate iodide (I^-) with several other tissues, including salivary gland, gastric mucosa, lactating mammary gland, choroid plexus, and ciliary body of the eye (1). Additionally, several normal and abnormal tissues, such as nonlactating mammary gland, lacrimal gland, nasopharynx, thymus, skin, placenta, inflammatory lung tissue, and lung carcinoma (7–13), have been reported to take up radioiodine. The iodide transport system in these extrathyroidal tissues reveals several functional similarities to its thyroid counterpart, such as inhibition of iodide transport by thiocyanate and perchlorate

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(1). By contrast, extrathyroidal iodide transport is not regulated by TSH, and extrathyroidal tissues are not able to organify the accumulated I^- (1). Our current study was designed to evaluate, using Northern blot analysis and RT-PCR amplification followed by Southern hybridization, the distribution and abundance of hNIS gene expression in a broad range of human extrathyroidal tissues. Further, we investigated whether the lower levels of iodide transport and concentrating activity in extrathyroidal tissues may be caused by expression of an altered hNIS protein structure. To this purpose, the entire coding region of the hNIS gene was amplified and cloned from parotid gland, mammary gland, and gastric mucosa and was compared with the recently published thyroid-derived hNIS gene sequence.

Materials and Methods

hNIS-cDNA probe preparation

Total RNA was isolated from human thyroid tissue, and single-stranded oligo-(dT)-primed cDNA was generated. The cDNA probes for Northern blot analysis were generated by PCR using a pair of hNIS gene-specific primers (sense primer: residues 1184–1202; antisense primer: residues 1648–1667). To facilitate subcloning of PCR-products, primers were designed to contain recognition sites for the restriction enzymes *Eco*RI and *Bam*HI, respectively. Following amplification, PCR products were digested with *Bam*HI and *Eco*RI (at 37 C, for 1.5 h) and were separated on an ethidium bromide-stained agarose gel. The band of interest was excised and purified using the QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany). This fragment was ligated overnight at 16 C into the *Bam*HI/*Eco*RI-sites of the DNA-plasmid pBluescript-SK II (−) (Stratagene, Heidelberg, Germany). Transfection of competent *Escherichia coli* XL1-blue cells was performed by electroporation using the Easyject Plus Electroporation System (Eurogentec, Seraing, Belgium). Clone phNIS-1 was obtained and plasmid DNA was purified with Qiagen-tip 20 (Qiagen), followed by sequencing using Sequenase version 2.0 (U.S. Biochemicals, Bad Homburg, Germany) and [α-^{35}S]dATP for labeling. For Northern blot analyses, the hNIS-1 fragment was cut out of the vector, resolved on an ethidium bromide-stained agarose gel, and purified using QIAquick Gel Extraction Kit (Qiagen).

Northern blot analysis of hNIS messenger RNA (mRNA)

Total RNA was isolated from various human tissue specimens that had been obtained at surgery, endoscopy, or autopsy, and they were immediately frozen in liquid nitrogen. Thereafter, RNA was electrophoresed on a 1% agarose gel containing 2 M formaldehyde, and was transferred overnight in 20 × saline-sodium citrate (SSC) to a positively charged nylon membrane (Qiagen). In addition, multiple tissue Northern blots were obtained from Clontech Laboratories, Inc. (Heidelberg, Germany). The hNIS-gene specific cDNA-fragment was radiolabeled with [α-^{32}P]deoxyadenosine-5′-triphosphate by random priming (Amersham, Braunschweig, Germany), and was used as a hybridization probe. Blots were prehybridized at 42 C in a hybridization mix containing 50% formamide for 6 h, followed by hybridization at 42 C for 16 h. Blots were then rinsed four times in 2x SSC/0.05% SDS at room temperature for 10 min, and twice in 0.1x SSC/0.1% SDS at 50 C for 20 min, respectively. Exposures were made at −80 C for 48 h using Kodak X-OMAT AR film sigma, (Deisenhofen, Germany). To strip off the hNIS cDNA probe, blots were treated in 0.5% SDS at 95 C for 10 min and were reprobed with a human β-actin cDNA probe to monitor RNA integrity and quantity. Computer-assisted densitometric analysis of band intensities was performed, and hNIS-measurements were normalized for β-actin signal intensity.

RNA preparation and PCR amplification

Total RNA was isolated from a broad range of normal human tissues by the modified acid guanidinium thiocyanate-phenol-chloroform method, according to Chomczynski and Sacchi (14), using the RNeasy Midi Kit (Qiagen). Pooled mRNA from various types of salivary glands was purchased from Clontech. Single-stranded oligo (dT)-primed cDNA was generated using Superscript II reverse transcriptase (Life Technologies, Eggenstein, Germany). Oligonucleotide primers, used for analysis of hNIS RNA expression, were CCC GGA TCC GCT GGC CCT GCT CAT CAA (nucleotides 1184–1202: sense strand) and CCC GAA TTC GCA GGC CGG CAG GAA CAT TC (nucleotides 1648–1667: antisense strand) (cDNA sequences underlined). These primers should generate a product that is 483 bp in length. Amplification was performed with 3 μL of each cDNA template, 50 pmol of each primer in 5 μL 10 × reaction buffer (Boehringer Mannheim, Mannheim, Germany), and 2 U *Taq* DNA polymerase (Boehringer Mannheim) in a final vol of 50 μL. The amplification reaction was for 30 cycles, and each cycle consisted of 94 C for 1 min (denaturation), 58 C for 2 min (annealing), and 72 C for 2 min (extension), followed by a final 10 min elongation at 72 C.

To control integrity of the cDNA templates and to rule out DNA contamination carried over in the samples, all templates were amplified with primers that span an intron of the β-actin gene. Oligonucleotide primers for β-actin amplification were TGA CGG GGT CAC CCA CAC TGT GCC CAT CTA (nucleotides 1038–1067: sense-strand) and CTA GAA GCA TTG CGG TGG ACG ATG GAG GG (nucleotides 1876–1905: antisense-strand). The expected β-actin product from a cDNA template is 661 bp. Reaction conditions were as above. In addition, to rule out the possibility of illegitimate transcription, all cDNA samples were amplified with a pair of intron-spanning primers designed to amplify 683-bp and 512-bp fragments of the human thyroglobulin gene. Oligonucleotide primers for thyroglobulin amplification were GGC CTG TGT CCC ATG TCC TG (sense strand) and TGG CTC CTG AGG CTG AGA AC (antisense strand) (15). All amplifications were run at cycle numbers that ensured linear amplification (30 cycles for NIS and thyroglobulin amplifications and 25 cycles for β-actin amplification).

Southern blot analysis

Ten microliters of each PCR product were separated on 1% agarose gels, stained with ethidium bromide, and transferred to positively charged nylon membranes (Qiagen). Hybridization was carried out with a digoxigenin (DIG)-labeled internal hNIS-specific oligonucleotide probe (nucleotides 1460–1477). Membranes were washed with buffer 1 (0.1 mol/L maleic acid, 0.15 mol/L NaCl; pH 7.5) containing 0.3% (vol/vol) Tween-20 and then incubated in buffer 2 [1% blocking solution (Boehringer Mannheim) in buffer 1] for 30 min. After incubation in anti-DIG-alkaline phosphatase-conjugate (dilution 1:10.000 in buffer 2) for 30 min, membranes were washed twice in buffer 1 containing 0.3% Tween-20 and then equilibrated in buffer 3 (0.1 mol/L Tris-HCl, 0.1 mol/L NaCl, 50 mmol/L $MgCl_2$). Membranes were then incubated in lumigen-PPD (dilution 1:100 in buffer 3) for 5 min and exposed to x-ray film for 10 min.

Amplification and subcloning of hNIS-specific cDNA from extrathyroidal tissues

Total RNA from parotid gland and gastric mucosa from normal human donors was prepared as described above. mRNA from mammary gland was purchased from Clontech. Single-stranded oligo-(dT)-primed cDNAs were generated and used as templates for cyclic thermal amplification with the EXPAND High Fidelity PCR System (Boehringer Mannheim). This system's proofreading activity markedly decreases the possibility of a point mutation caused by *Taq* amplification. cDNA fragments representing the entire coding region of the hNIS gene were then generated by PCR using three overlapping pairs of hNIS-specific primers (hNIS-M1-hNIS-M6). Table 1 illustrates nucleotide sequences of these primers, as well as their localizations and orientations, respectively. To facilitate subcloning of specific PCR products, primers were designed to contain recognition sites for the restriction enzymes *Eco*RI and *Bam*HI, respectively. Restriction hydrolysis of the resulting PCR products using *Bam*HI and *Eco*RI, isolation, ligation into DNA-plasmid pBluescript-SK II (−), and transformation were performed as described above. Fig. 1 shows a schematic illustration of the amplification and cloning strategy. Plasmid DNA of at least two independent cDNA clones, obtained from each tissue, were isolated and sequenced using the RR DyeDeoxy Terminator Cycle Sequencing Kit (Perkins Elmer Applied Biosystems, Weiterstadt, Germany).

TABLE 1. Primers used for PCR amplification

Primer	Sequence 5′ to 3′	Location (5)	Direction
hNIS-M1	TAATACGACTCACTATAGGGAGAATTCTTGAGCACGCAGGGCGTC	nt. 297-315	Sense
hNIS-M2	ATTTAGGTGACACTATAGGAGGATCCGCCACTTAGCATCACCACG	nt. 932-950	Antisense
hNIS-M3	TAATACGACTCACTATAGGGAGAATTCGGCATGAAGGCTGTGGTC	nt. 892-911	Sense
hNIS-M4	ATTTAGGTGACACTATAGGAGGATCCGCAGGCCGGCAGGAACATTC	nt. 1648-1667	Antisense
hNIS-M5	TAATACGACTCACTATAGGGAGAATTCGGCTCCTTCACCGTCATG	nt. 1590-1607	Sense
hNIS-M6	ATTTAGGTGACACTATAGGAGGATCCGTTCCATCCCAGGGTGTCAG	nt. 2300-2319	Antisense

The underlined region is complementary to a portion of the hNIS gene. nt., nucleotides.

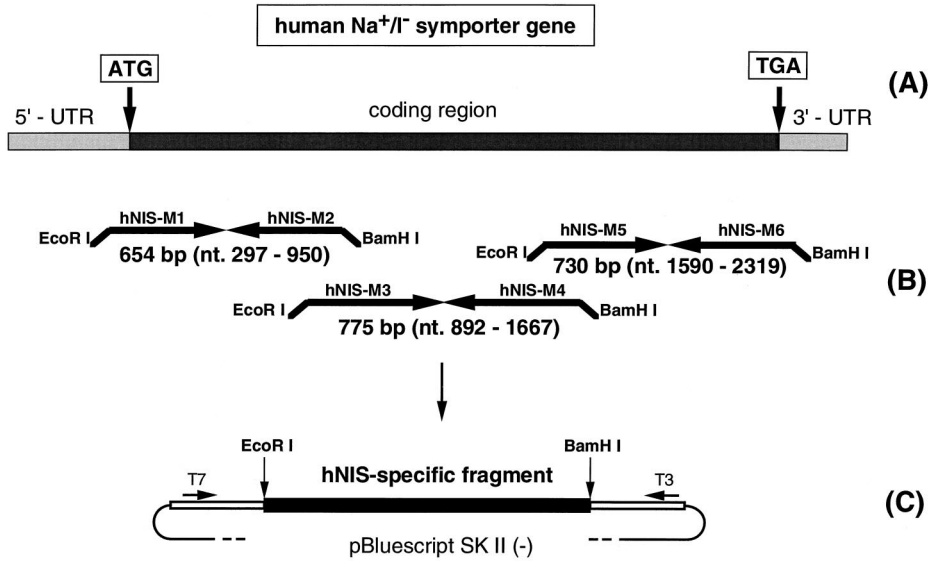

FIG. 1. Schematic illustration of the amplification and cloning strategy for the coding regions of hNIS gene expressed in parotid gland, mammary gland, and gastric mucosa. The entire coding region of NIS gene was amplified (A) using three pairs of highly specific overlapping pairs of primers (hNIS-M1/hNIS-M2, hNIS-M3/hNIS-M4, and hNIS-M5/hNIS-M6), designed to contain restriction sites for *Bam*HI and *Eco*RI, respectively (B). The resulting cDNA fragments of appropriate lengths (654 bp, 775 bp, and 730 bp, respectively) were digested with *Bam*HI and *Eco*RI, separated, isolated, and ligated into the *Bam*HI/*Eco*RI sites of DNA plasmid pBluescript-SK II (−). pBluescript-specific T3 and T7 primers, as well as hNIS specific internal primers, were employed for sequencing (C).

Results

Northern blot analysis

hNIS mRNA expression profiles were examined by high-stringency Northern blot analysis using a ^{32}P-labeled hNIS-specific cDNA probe. Abundant hNIS mRNA expression was detectable as a single species of approximately 4 kb in thyroid gland. In addition, a weaker hNIS mRNA signal was also detectable in parotid gland (Figs. 2 and 3). Even after film exposure for 1 week, strong β-actin mRNA signals (but not an hNIS mRNA signal) were detected in brain, thymus, heart, stomach, small intestine, colon, lung, liver, skeletal muscle, kidney, spleen, placenta and peripheral blood leucocytes, and in a variety of endocrine tissues, such as pancreas, adrenal gland, prostate, testis, and ovary (data not shown).

PCR amplification and Southern blot analysis

For analysis of low levels of hNIS gene expression in small samples obtained from various normal human tissues, total RNA was prepared from freshly frozen tissue specimens, followed by RT-PCR with a pair of hNIS-specific oligonucleotide primers (Figs. 4 and 5, *top*). To monitor cDNA integrity and quantity, all samples were coamplified with a pair of human β-actin primers (Figs. 4 and 5, *middle*). Moreover, to rule out the possibility of contaminating DNA and illegitimate transcription, all cDNA samples were coamplified with a pair of primers specific for human thyroglobulin. Human thyroglobulin-specific transcripts were detected in thyroid tissue but not in any other cDNA sample examined. hNIS-specific transcripts were confirmed by Southern hybridization using a DIG-labeled internal hNIS-specific oligonucleotide probe (Figs. 4 and 5, *bottom*). RT-PCR and Southern hybridization revealed hNIS gene expression in thyroid gland, salivary gland, parotid gland, submandibular gland, pituitary gland, pancreas, testis, mammary gland, gastric mucosa, prostate and ovary, adrenal gland, heart, thymus, and lung. By contrast, hNIS transcripts were not detected in normal orbital fibroblasts, colon, and nasopharyngeal mucosa (Figs. 4 and 5).

hNIS cDNA subcloning

To investigate whether the reduced capacity to trap iodide in extrathyroidal tissues, compared with thyroid gland, may be caused by an altered primary structure of hNIS cDNA, single-stranded cDNAs were generated from RNA derived from parotid gland, mammary gland, and gastric mucosa. To facilitate PCR cloning and sequencing, amplifications were performed using three overlapping NIS-specific pairs of primers designed to encompass the entire coding region of

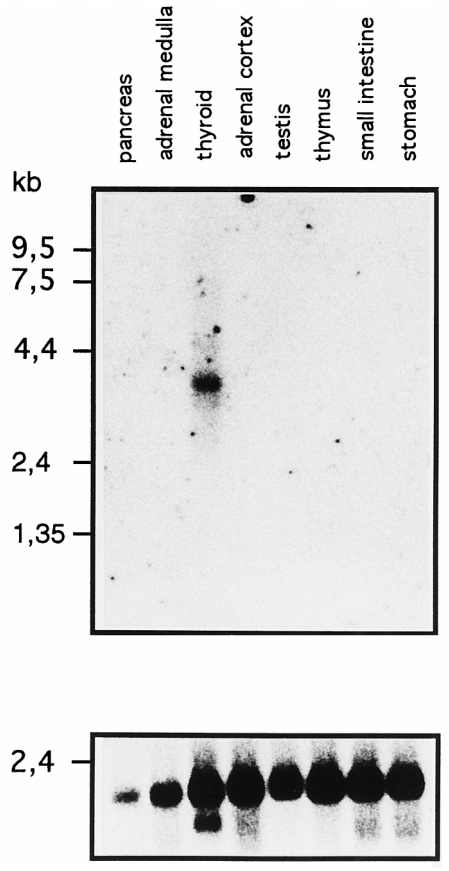

FIG. 2. Northern blot analysis of mRNA derived from several normal human tissues using a ^{32}P-labeled hNIS-specific cDNA probe. hNIS mRNA was detected as a single species of approximately 4 kb in thyroid gland. No hNIS mRNA signal was detectable in pancreas, adrenal gland, testis, thymus, small intestine, and stomach. Hybridization with a β-actin probe served as a control.

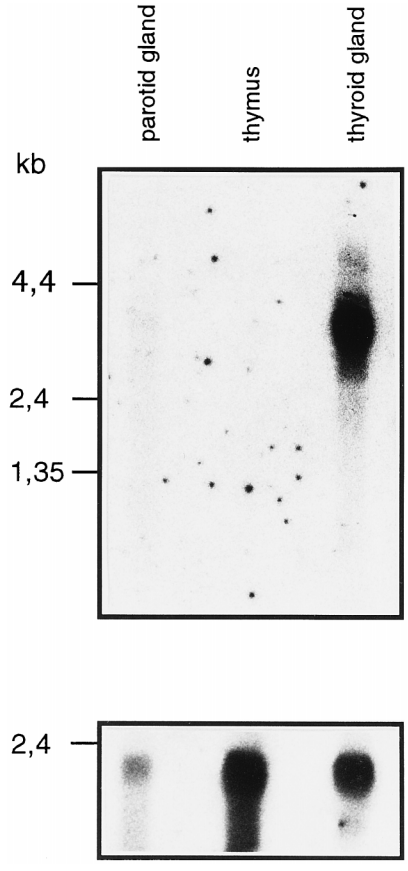

FIG. 3. Northern blot analysis of mRNA derived from various normal human tissues using a ^{32}P-labeled hNIS-specific cDNA probe. Abundant hNIS mRNA expression was detected as a single species of approximately 4 kb in thyroid gland. A weaker hNIS mRNA signal was detectable in parotid gland. No hNIS mRNA signal was detected in thymus. Hybridization with a β-actin probe served as a control.

the hNIS gene. PCR products of appropriate lengths (654 bp, 775 bp, and 730 bp, respectively) were obtained from each tissue, followed by subcloning and sequencing of at least two independent cDNA clones derived from each tissue. Nucleotide sequences of hNIS cDNAs, expressed by each of the three extrathyroidal tissues, revealed full identity with the recently published human thyroid-derived NIS cDNA (5). No point mutations, insertions, deletions, or evidence of alternative splicing were found.

Discussion

The recent cloning and characterization of the NIS (2–5, 16, 17) has made it possible to investigate, at the molecular level, the complex mechanisms involved in the regulation of iodide uptake by thyroidal and nonthyroidal tissues. In addition to the iodide concentrating activity of the thyroid gland, iodide uptake is a characteristic feature of several extrathyroidal tissues, such as salivary glands, gastric mucosa, lactating mammary gland, choroid plexus, ciliary body of the eye, skin, and placenta (1, 13). Furthermore, both cerebrospinal fluid and aqueous humor have been reported to exhibit low concentrations of I^- (1). The extraction of I^- that reaches the cerebrospinal fluid by slow diffusion has been shown to be mediated by an active I^- translocating mechanism in the choroid plexus (1). A similar mechanism seems to be at work in the ciliary body of the eye (1). Moreover, in the ducts of salivary glands, an active I^- transport system provides for a higher concentration of I^- in saliva, compared with plasma (1). Consequently, radioiodine (^{131}I) therapy may produce significant damage to salivary glands, resulting in salivary dysfunction in a significant number of patients, most likely as a result of radioiodine uptake and concentration in the striated ducts of the salivary glands (9). Similarly, I^- is actively transported from plasma to gastric secretions by a I^- translocating system (1). Also, lactating breast tissue is capable of concentrating I^- in milk, thereby supplying I^- to the newborn (1). Despite important differences between the iodide transport system in nonthyroidal and thyroidal tissues, such as lack of regulation by TSH and inability to organify accumulated I^- in nonthyroidal tissues, a genetic link between the I^- transport systems in thyroid gland and extrathyroidal tissues is suggested by several reports of patients suffering from the simultaneous genetic absence of I^- transport in thyroid, salivary glands, and gastric mucosa (1). In support of these clinical observations, we found substantial quantities of NIS gene expression in salivary glands, mam-

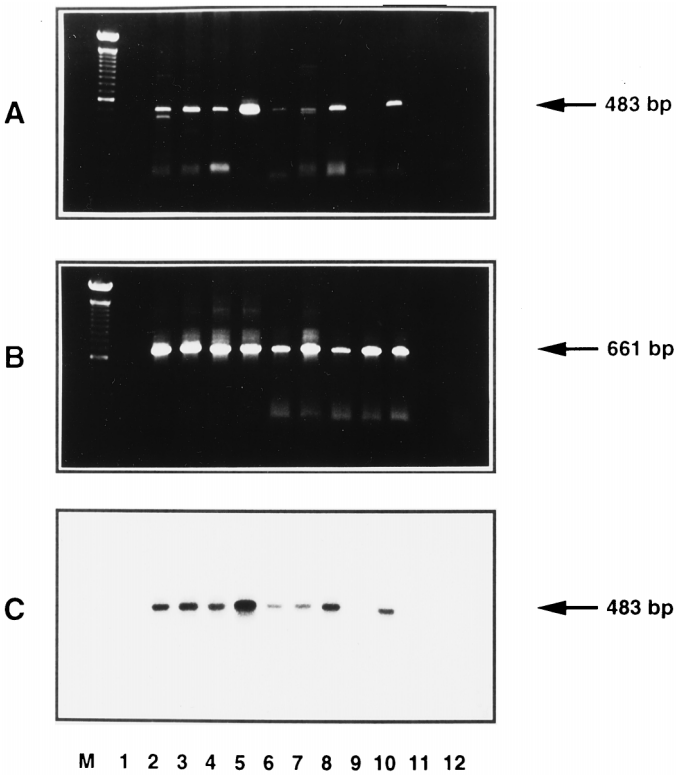

FIG. 4. PCR amplification of cDNA derived from various normal human tissues using a pair of hNIS-specific oligonucleotide primers designed to amplify a 483-bp fragment of the hNIS gene (A). All samples were coamplified with a pair of β-actin oligonucleotide primers designed to amplify a 661-bp fragment of the β-actin gene (B). hNIS-specific transcripts were confirmed by Southern hybridization using a DIG-labeled internal hNIS-specific oligonucleotide probe (C). Lane M, 100-bp ladder; lane 1, blank; lane 2, thymus; lane 3, pituitary gland; lane 4, mammary gland; lane 5, salivary gland; lane 6, submandibular gland; lane 7, parotid gland; lane 8, stomach; lane 9, colon; lane 10, thyroid gland; lane 11, blank; lane 12, negative control (no cDNA).

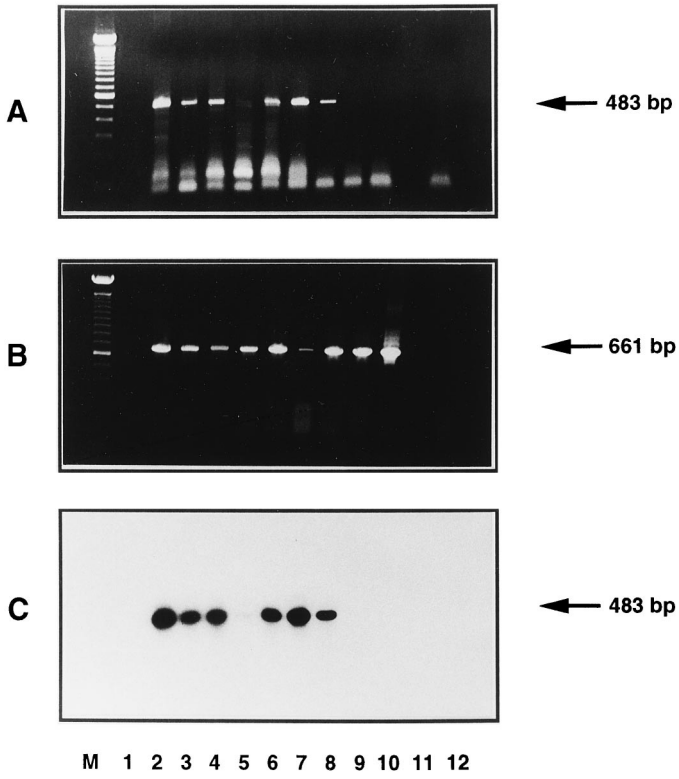

FIG. 5. PCR amplification of cDNA derived from various normal human tissues with a pair of hNIS-specific oligonucleotide primers designed to amplify a 483-bp fragment of the hNIS gene (A). All samples were coamplified with a pair of β-actin oligonucleotide primers designed to amplify a 661-bp fragment of the β-actin gene (B). hNIS-specific transcripts were confirmed by Southern hybridization using a DIG-labeled internal hNIS-specific oligonucleotide probe (C). Lane M, 100-bp ladder; lane 1, blank; lane 2, testis; lane 3, prostate; lane 4, ovary; lane 5, lung, lane 6, adrenal gland; lane 7, pancreas; lane 8, heart; lane 9, nasopharynx; lane 10, normal orbital fibroblasts; lane 11, blank; lane 12, negative control (no cDNA).

mary gland, and gastric mucosa, indicating that the NIS gene product may control iodide uptake by these tissues.

Whole-body scintigraphy with radioiodine-131, an important diagnostic test in the management of patients with differentiated thyroid cancer, has revealed false-positive uptake in a significant number of cases. Radioiodine uptake has been noted in a variety of normal and abnormal extrathyroidal tissues, such as nonlactating mammary gland (8), lacrimal gland (9), nasopharynx (10), thymus (11), skin (13), placenta (13), pleuropericardial (7) and renal cysts (10), inflammatory lung disease (10), Meckel's diverticulum (10), ovarian cystadenoma (10), meningeoma (10), and lung carcinoma (12). Although iodide uptake by these extrathyroidal tissues may result from nonspecific iodide accumulation, our detection of significant quantities of hNIS gene expression in thyroid gland, salivary glands, thymus, pituitary gland, pancreas, testis, mammary gland, and gastric mucosa, and of lower degrees of NIS gene expression in prostate, ovary, adrenal gland, lung, heart, and nasopharyngeal mucosa suggests that iodide transport in some of these tissues may be a specific property conferred by the expression of NIS. Recently, in support of our study, Smanik and colleagues (6) have detected hNIS expression in breast, colon, and ovary by RT-PCR, suggesting that hNIS may mediate the iodide uptake activity in both thyroid and these nonthyroid tissues. In contrast to their observation, perhaps because of variable levels of hNIS gene expression in different colon segments or interindividual differences in hNIS gene expression, we were not able to detect hNIS gene expression in colon.

Compared with the thyroid gland, iodide transport and concentrating activity is much lower in extrathyroidal tissues. Reduced iodide uptake activity in these tissues may result from expression of a hNIS protein with an altered primary structure. Recently, several hNIS gene mutations have been detected in patients with congenital hypothyroidism caused by an iodide transport defect (18–20). To examine whether an altered primary structure of NIS in extrathyroidal tissues may explain their diminished iodide uptake capacity, we determined the entire hNIS coding sequence in several extrathyroidal tissues. Our results demonstrate that, in extrathyroidal tissues, reduced iodide trapping activity does not seem to be caused by an altered NIS cDNA structure, because NIS coding sequences in tissues such as parotid gland, mammary gland, and gastric mucosa revealed full identity with thyroid hNIS cDNA. Moreover, no evidence of alternative splicing of NIS gene was found in the tissues analyzed. Because identical NIS proteins are en-

coded in thyroid tissue and extrathyroidal tissues, diminished iodide transport in extrathyroidal tissues may result from altered NIS gene transcriptional activity, perhaps as a consequence of altered promoter structure or function, or from altered NIS mRNA or protein turnover. Variable and lower NIS transcriptional activity in extrathyroidal tissues may be accounted for, at least in part, by thyroid-specific transcription factors that act on the NIS promoter to control NIS gene expression in the thyroid gland. Specific gene expression of thyroglobulin, thyroperoxidase, and TSH-receptor in the thyroid gland is well known to be regulated at the transcriptional level (21). Thyroid transcription factor 1 (TTF-1), a homeodomain-containing protein, has been found to bind to all three thyroid-specific promoters and to activate their transcriptional activity (22, 23). Recently, cloning and functional analysis of rat NIS gene has identified a binding sequence for TTF-1 within the rat NIS promoter region (16). These findings suggest that TTF-1 may be one of the factors capable of activating NIS gene expression in the thyroid gland, thus accounting for lower levels of NIS gene expression in extrathyroidal tissues. Further studies will address this hypothesis and examine the mechanisms of tissue-specific NIS gene expression.

Recently, several lines of evidence have identified hNIS as a potential autoantigen in autoimmune thyroid diseases. Serum obtained from a patient with Hashimoto's thyroiditis, autoimmune gastritis, and rheumatoid arthritis was found to inhibit the chronic TSH-induced I^- uptake by cultured dog thyrocytes, suggesting the presence of autoantibodies against hNIS in this patient's serum (24). After cloning of rNIS, immunoblot analysis of sera from patients with Graves' disease and patients with Hashimoto's thyroiditis has indicated that autoantibodies against NIS are commonly detected in patients with autoimmune thyroid diseases (25). Interestingly, NIS autoantibodies in sera from patients with Hashimoto's thyroiditis were found to inhibit iodide transport by thyrocytes (26). Moreover, Morris and colleagues (27, 28) have observed increased binding of IgG, from patients with autoimmune thyroid diseases, to several synthetic rNIS and hNIS peptides. Taken together, there is increasing evidence that sera from patients with Hashimoto's thyroiditis and Graves' disease contain antibodies that cross-react with rNIS and hNIS. Thus, NIS seems to represent a potential autoantigen that may play a role in the pathogenesis of autoimmune thyroid disease and associated autoimmune diseases. In fact, in various extrathyroidal tissues, such as gastric mucosa, salivary glands, and lacrimal gland, hNIS may act as a target antigen for T cells and cross-reacting autoantibodies, thus perhaps providing a link between autoimmune thyroid diseases and associated autoimmune diseases of other organ systems, such as autoimmune gastritis and Sjögren's syndrome. Given the expression of hNIS mRNA in various endocrine and secretory glands that are common targets in autoimmune diseases, NIS protein expression in these tissues and its potential role as a shared autoantigen in the evolution of polyglandular autoimmune syndromes warrants further analysis. With the recent availability of specific antibodies generated against hNIS protein, these studies are now in progress.

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