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Escape from the Acute Wolff-Chaikoff Effect Is Associated with a Decrease in Thyroid Sodium/Iodide Symporter Messenger Ribonucleic Acid and Protein*

PETER H. K. ENG, GUEMALLI R. CARDONA, SHIH-LIEH FANG, MICHAEL PREVITI, SHARON ALEX, NANCY CARRASCO, WILLIAM W. CHIN, AND LEWIS E. BRAVERMAN

Division of Genetics (P.H.K.E., G.R.C., S.-L.F., M.P., S.A., W.W.C., L.E.B.), Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts 02115; and Department of Molecular Pharmacology (N.C.), Albert Einstein College of Medicine, Bronx, New York 10461

ABSTRACT

In 1948, Wolff and Chaikoff reported that organic binding of iodide in the thyroid was decreased when plasma iodide levels were elevated (acute Wolff-Chaikoff effect), and that adaptation or escape from the acute effect occurred in approximately 2 days, in the presence of continued high plasma iodide concentrations. We later demonstrated that the escape is attributable to a decrease in iodide transport into the thyroid, lowering the intrathyroidal iodine content below a critical inhibitory threshold and allowing organification of iodide to resume. We have now measured the rat thyroid sodium/iodide symporter (NIS) messenger RNA (mRNA) and protein levels, in response to both chronic and acute iodide excess, in an attempt to determine the mechanism responsible for the decreased iodide transport. Rats were given 0.05% NaI in their drinking water for 1 and 6 days in the chronic experiments, and a single 2000-μg dose of NaI ip in the acute experiments. Serum was collected for iodine and hormone measurements, and thyroids were frozen for subsequent measurement of NIS, TSH receptor, thyroid peroxidase (TPO), thyroglobulin, and cyclophilin mRNAs (by Northern blotting) as well as NIS protein (by Western blotting). Serum T_4 and T_3 concentrations were significantly decreased at 1 day in the chronic experiments and returned to normal at 6 days, and were unchanged in the acute experiments. Serum TSH levels were unchanged in both paradigms. Both NIS mRNA and protein were decreased at 1 and 6 days after chronic iodide ingestion. NIS mRNA was decreased at 6 and 24 h after acute iodide administration, whereas NIS protein was decreased only at 24 h. TPO mRNA was decreased at 6 days of chronic iodide ingestion and 24 h after acute iodide administration. There were no iodide-induced changes in TSH receptor and thyroglobulin mRNAs. These data suggest that iodide administration decreases both NIS mRNA and protein expression, by a mechanism that is likely to be, at least in part, transcriptional. Our findings support the hypothesis that the escape from the acute Wolff-Chaikoff effect is caused by a decrease in NIS, with a resultant decreased iodide transport into the thyroid. The observed decrease in TPO mRNA may contribute to the iodine-induced hypothyroidism that is common in patients with Hashimoto's thyroiditis. (*Endocrinology* **140:** 3404–3410, 1999)

AUTOREGULATION IN THE thyroid refers to the regulation of iodine metabolism within the thyroid gland, independent of TSH. It was first reported by Morton *et al*. (1) in 1944, who observed that large amounts of iodide inhibited the formation of thyroid hormones by incubated sheep thyroid slices. Wolff and Chaikoff (2) then reported that organic binding of iodide within the rat thyroid was blocked when the plasma iodide level achieved a critical threshold. This inhibition defines the Wolff-Chaikoff effect. They next demonstrated that this inhibitory effect of excess iodide was transient, lasting from 26–50 h, and that the thyroid escaped or adapted to prolonged iodide excess, resuming near-normal hormone synthesis (3). The mechanism responsible for the acute Wolff-Chaikoff effect remains elusive and has been postulated to be caused by organic iodocompounds formed within the thyroid (4). α-Iodohexadecanal, a major iodolipid, has been shown to inhibit nicotinamide adenine dinucleotide phosphate oxidase, thyroid peroxidase (TPO), and TSH-induced cAMP formation in the thyroid and thus may be a potential mediator of the effect. The so-called escape phenomenon, however, has been less well studied. Braverman and Ingbar suggested that adaptation to the acute Wolff-Chaikoff effect was caused by a decrease in iodide transport into the thyroid, which reduced the intrathyroidal iodide to concentrations that were insufficient to sustain the decreased organification of iodide (5). Recently, the complementary DNA (cDNA) encoding the protein responsible for the active transport of iodide from blood to thyroid was cloned by Dai *et al.* (6). This polytopic membrane protein, expressed in thyroid follicular cells, is termed the sodium-coupled iodide cotransporter or sodium/iodide symporter (NIS). We hypothesized that changes in NIS expression might account for iodide autoregulation in the thyroid. In the present study, we reexamined the mechanism responsible for the escape from the acute Wolff-Chaikoff effect by determining NIS messenger RNA (mRNA) and protein levels in rat thyroids in response to acute and prolonged administration of excess iodide.

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Address all correspondence and requests for reprints to: Peter Eng, M.D., Division of Genetics, Department of Medicine Brigham and Women's Hospital, 75 Francis Street, Thorn 1013, Boston, Massachusetts 02115. E-mail: eng@rascal.med.harvard.edu.
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Materials and Methods

Animals

Sprague Dawley male rats (200–250 g; Charles River Laboratories, Inc., Kingston, NY) were used in all of the experiments and were maintained on Lab Diet no. 5001, PMI International (Brentwood, MO) and water *ad libitum*.

The chronic excess iodide experiment was carried out as follows: The rats were divided into 3 groups: control and 1 and 6 days of iodide excess. Rats were given either distilled water (control group) or 0.05% iodide, as NaI, in the drinking water for 1 or 6 days. The addition of iodide to the drinking water was timed so that all the rats were killed on the same day. The thyroid gland from each rat was removed immediately, snap frozen, and stored at −70 C for measurement of specific RNA and proteins. Blood from each rat was collected, and the serum was frozen for later measurement of serum iodide, TSH, T_3, and T_4 concentrations. There was a total of 48 rats, with 16 rats in each group. Three separate experiments were carried out using the same protocol. The first experiment had 8 rats in each group, and the second and third experiments had 4 rats in each group. The results of the 3 experiments were pooled for the purpose of statistical analysis.

The acute excess iodide experiment was carried out as follows: Rats were divided into 5 groups: control and 1, 2, 6, and 24 h after an acute iodide load. There were 4 rats in each group. A single ip injection of 2000 μg NaI in 0.5 ml saline was administered to the 1-, 2-, 6-, and 24-h groups before being killed. Saline alone was injected into the control group. Rats were killed, and their thyroid glands and blood were obtained as described above. A similar protocol was repeated with only 3 groups of rats (6 rats in each group): control and 6 and 24 h after iodide administration. The results of the 2 experiments were pooled for statistical analysis.

This study was approved by the Harvard Area Standard Committee on Animals, and it conforms with federal and state regulations governing the use of laboratory animals.

Serum iodine and hormone measurements

Serum iodine concentrations were assayed according to the modified Sandell-Koltoff method of Benotti and Benotti (7). Serum thyroid hormones, T_3 and T_4, and TSH concentrations were measured in duplicate by RIAs, in random order and in the same assay for each experiment. Serum TSH was measured by RIA using materials obtained from the National Pituitary Agency, NIH (Bethesda, MD). Serum T_3 and T_4 concentrations were determined by species-adapted RIAs using a single antibody technique with polyethylene glycol precipitation (8).

Thyroid RNA analysis

Total thyroid RNA was extracted using a commercial kit (RNeasy; QIAGEN, Inc., Chatsworth, CA). Northern analysis of the RNA was carried out as follows. Total RNA (10 μg/lane) was subjected to electrophoresis for 3 h in 1% agarose containing formaldehyde in 1× 4-morpholinopropanesulfonic acid. The RNA was then transferred overnight to a nylon membrane (Nytran; Schleicher & Schuell, Inc., Keene, NH) by diffusion blotting, and was UV cross-linked. The membrane was hybridized sequentially with five rat cDNA probes: NIS, TSH receptor (TSHr), TPO, thyroglobulin (Tg), and cyclophilin. Cyclophilin mRNA was used for normalization of the levels of thyroid mRNAs. The cDNA probes were labeled with [α-^{32}P]deoxycytidine 5′-triphosphate using a random primer protocol (Prime-It II Random Primer Labeling Kit; Stratagene, La Jolla, CA) to a specific activity of 0.2–2 × 10^9 cpm/μg DNA. Purified cDNA inserts (TSHr and TPO were provided by L. D. Kohn, National Institute of Diabetes and Digestive and Kidney Diseases, NIH (Bethesda, MD); and Tg by G. Vassart, Institut de Recherche Interdisciplinaire, Bruxelles, Belgium) were used as the probes. The membrane was prehybridized 1–2 h at 42 C, followed by an overnight hybridization at 42 C with the radiolabeled probes. The membrane was washed twice with 6× SSPE (NaCl, $NaPO_4$, EDTA)/0.5% SDS at room temperature, twice with 1× SSPE /0.5% SDS at 37 C, and (if high stringency was required) a further wash with 0.1× SSPE/0.1% SDS at 60 C. The membrane was exposed to a phosphorimager screen for an appropriate length of time, and the signal intensity was analyzed by a phosphorimaging system (Molecular Dynamics, Inc., Sunnyvale, CA).

Thyroid protein analysis

Total protein from the thyroid glands was extracted as follows. Frozen thyroid glands were thawed in RIPA buffer (1× PBS, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS) and protease inhibitors (aprotinin, sodium orthovanadate, and phenylmethylsulfonylflouride) were added. Homogenization was performed using a mechanical device (Polytron; Kinematica, Luzern, Switzerland). The homogenate was centrifuged at 15,000 × *g*, and the supernatant (which contained the whole cell lysate) was quantified spectrophotometrically using a modified Lowry method (Dc Protein Assay; Bio-Rad Laboratories, Inc., Hercules, CA). Western blot analysis was carried out as follows: Twenty-five micrograms of protein per lane were loaded on an 8% SDS polyacrylamide gel and subjected to electophoresis at a constant voltage (150 V). Electroblotting to a nitrocellulose membrane (Protran; Schleicher & Schuell, Inc.) was performed for 2 h at 90 mA using a semidry electroblotting system (MultiphorII Electrophoresis System; Pharmacia LKB Biotechnology, Uppsala, Switzerland). Blocking was done overnight using TTBS/milk (TBS, 1% Tween 20 and 5% milk). The membrane was incubated with a 1:5000 dilution of affinity-purified anti-NIS antibody in TTBS/milk. Two 5-min and two 15-min washes in TTBS were then performed. The membrane was incubated with a 1:25,000 dilution of a horseradish peroxidase conjugated antirabbit antibody (Pierce Chemical Co., Rockford, IL) in TTBS/milk. Two 5-min and two 15-min washes were again performed. The membrane was then incubated with an enhanced chemiluminescent substrate (Supersignal Substrate Western Blotting, Pierce Chemical Co.) and exposed to film. Quantitation of the signal intensity was performed by densitometry (Molecular Dynamics, Inc.).

Statistical analysis

Statistical analysis was performed using a statistical analysis program (Instat v 2.02; GraphPad Software, Inc., San Diego, CA). Comparison between groups was by ANOVA, followed by Tukey-Kramer test for intergroup comparison. Results are expressed as mean ± SE.

Results

We first performed experiments to determine the effect of chronic excess oral iodide administration on thyroid mRNA levels, especially NIS, over a period of 6 days.

Chronic iodide ingestion

Serum iodine and hormone levels. Serum iodine levels, after 1 and 6 days of iodide administration, were markedly elevated (more than 60-fold), compared with values in the control group (Table 1). There was no statistical difference in the serum iodine levels between the 1-day and 6-day groups. Serum T_3 and T_4 concentrations were significantly decreased in the rats treated with iodide for 1 day, compared with values in the control rats. Serum T_3 concentrations, after 6 days of iodide administration, were similar to those in the

TABLE 1. Effect of chronic and acute iodide administration on serum iodine

Groups	n	Serum iodine concentrations (μg/dl) (mean ± SE)
Control	16	10 ± 1.4
1 day of iodide in drinking water[a]	16	1915 ± 161
6 days of iodide in drinking water	16	1688 ± 133
Control	10	7.3 ± 0.3
1 h after ip iodide injection[b]	4	1314 ± 145
2 h after ip iodide injection	4	594 ± 108
6 h after ip iodide injection	10	394 ± 29
24 h after ip iodide injection	10	129 ± 17

[a] 0.05% I (NaI) in the drinking water.
[b] 2,000 μg I (NaI) ip.

control rats; but T_4 concentrations, after 6 days of iodide administration, were significantly higher than 1-day and control rats. TSH concentrations were not significantly different from control values at 1 or 6 days after iodide administration, although values were slightly higher at 1 day (Fig. 1).

mRNA expression. All mRNA results were normalized to thyroid cyclophilin mRNA levels. The expression of NIS mRNA was significantly decreased, to approximately 55% of control values, after 1 day of iodide administration; and it decreased further, to approximately 40% of control values, at 6 days (Fig. 2). TPO mRNA was decreased significantly 6 days after iodide administration but not after 1 day (Fig. 3). In contrast, TSHr mRNA and Tg mRNA were unchanged during iodide administration (data not shown).

Protein expression. The level of NIS protein was determined by Western blot analysis. It was markedly decreased, 1 and 6 days after iodide administration, compared with values in the control rats (Fig. 4).

The results from the chronic iodine ingestion experiments revealed an effect of NIS mRNA and protein as early as 24 h. Thus, we performed another set of experiments to determine,

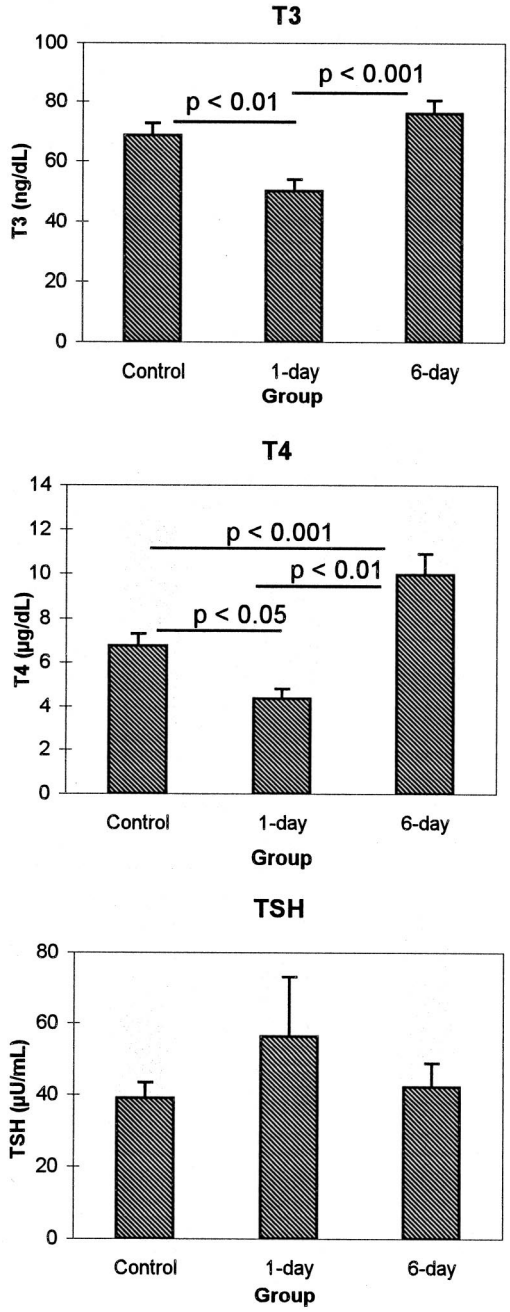

FIG. 1. Rat serum hormone levels during chronic iodide ingestion. Results are pooled from three separate experiments, with n = 16 rats in each group. Shown are the means ± SEM of each group. A P value of <0.05 is significant.

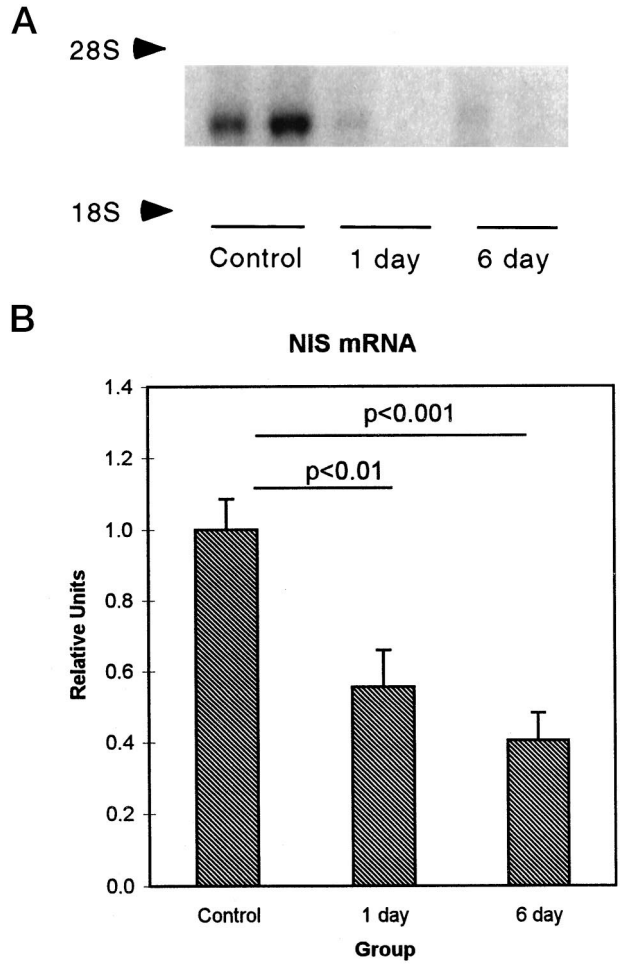

FIG. 2. Northern blot analysis of thyroid RNAs: chronic excess iodide. A, Autoradiograph of a representative Northern blot of thyroid RNA extracted from rats subjected to chronic excess iodide. The blot was hybridized with a ^{32}P-labeled NIS probe and exposed to film. Lanes 1 and 2 are controls, lanes 3 and 4 are from rats in the 1-day group, and lanes 5 and 6 are from rats in the 6-day group. B, Rat thyroid NIS mRNA levels, during chronic iodide ingestion, as determined by Northern blotting. Band density was measured using a Phosphorimager. Results are pooled from three separate experiments and are normalized, with respect to individual thyroid cyclophilin mRNAs. They are expressed as relative units, with the control group mean = 1 [control group (n = 10), 1-day group (n = 11), and 6-day group (n = 12)]. Shown are the means ± SEM of each group. A P value of <0.05 is significant.

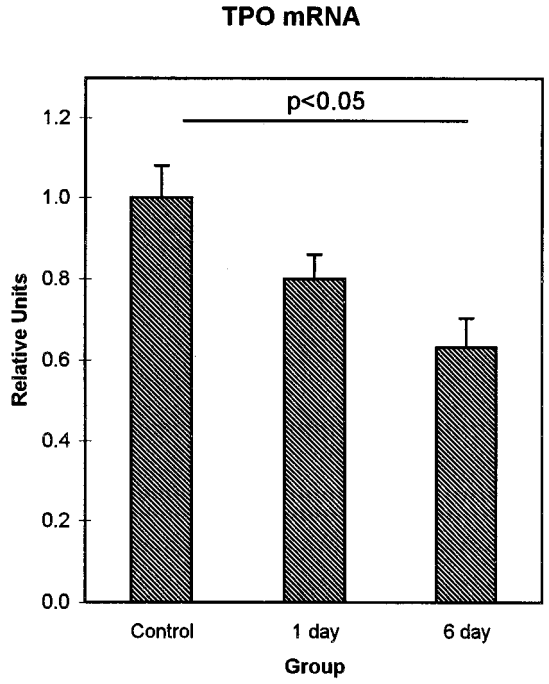

FIG. 3. Rat thyroid TPO mRNA levels during chronic iodide ingestion, as determined by Northern blotting. Band density was measured using a Phosphorimager. Results are pooled from three separate experiments and are normalized, with respect to individual thyroid cyclophilin mRNAs. They are expressed as relative units, with the control group mean = 1 [control group (n = 10), 1-day group (n = 7), and 6-day group (n = 7)]. Shown are the means ± SEM of each group. A P value of <0.05 is significant.

more accurately, when the change occurred. We administered a single ip injection of NaI and measured the levels of thyroid mRNAs and NIS protein at several time points, up to 24 h.

Acute iodide administration

Serum iodine and hormone levels. Serum iodine concentrations were markedly increased 1 h after iodide administration and progressively decreased at 2, 4, 6, and 24 h, but they were still greater than 100 μg/dl at 24 h (Table 1). There were no significant changes in serum T_3, T_4, or TSH concentrations at any time point after iodide administration (data not shown).

mRNA expression. The level of NIS mRNA was unchanged at 1 and 2 h after the acute administration of 2000 μg NaI ip but was significantly decreased at 6 h (60% of control) and further decreased at 24 h (40% of control) (Fig. 5). TPO mRNA was unchanged at 1, 2, and 6 h but was decreased at 24 h (Fig. 6). There were no statistical differences among the levels of TSHr or Tg mRNA at 1, 2, 6, and 24 h after the acute administration of iodide (data not shown).

Protein expression. The level of NIS protein, as determined by Western blot analysis, was unchanged 6 h after iodide administration but was markedly decreased to 30% of control values at 24 h (Fig. 7).

Discussion

The acute Wolff-Chaikoff effect and its escape occur during the exposure of the normal thyroid to high levels of

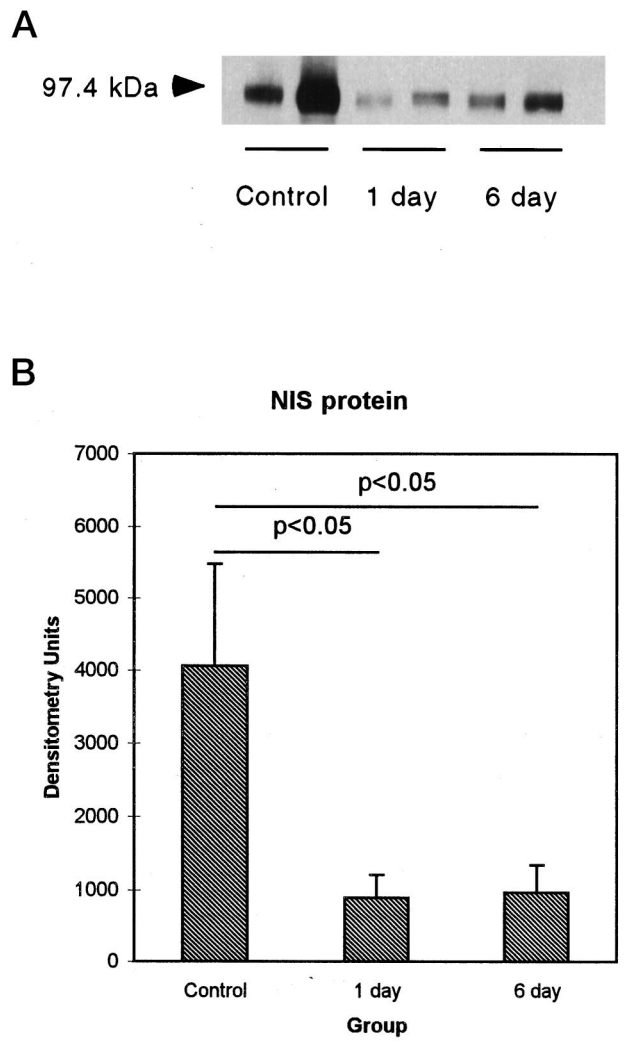

FIG. 4. Western blot analysis of NIS protein in thyroid tissue: chronic excess iodide. A, Autoradiograph of Western blot of thyroid protein extracted from rats subjected to chronic excess iodide. The blot was hybridized with a primary anti NIS-antibody and a secondary horseradish peroxidase-conjugated antibody. Protein detection was by enhanced chemiluminesence. Lanes 1 and 2 are controls, lanes 3 and 4 are from rats in the 1-day group, and lanes 5 and 6 are from rats in the 6-day group. The NIS protein is approximately 90 kDa. B, Rat thyroid NIS protein levels, during chronic iodide ingestion, as determined by Western blotting. Band density was measured using a densitometer. Results are from one experiment and are expressed as densitometry units [control group (n = 4), 1-day group (n = 4), and 6-day group (n = 4)]. Shown are the means ± SEM of each group. A P value of <0.05 is significant.

plasma iodide. In the present experiments, high concentrations of iodide in the circulation were achieved in normal rats by the administration of 0.05% NaI in the drinking water for 1 and 6 days or by a single ip injection of 2000 μg NaI. After 1 day of iodide ingestion, serum T_3 and T_4 concentrations significantly decreased and returned to normal after 6 days of continued iodide treatment. The decrease in the serum thyroid hormone concentrations, after 1 day, was possibly the result of both acute inhibition of iodide organification and subsequent thyroid hormone synthesis (the acute Wolff-Chaikoff effect) and acute inhibition of hormone release from

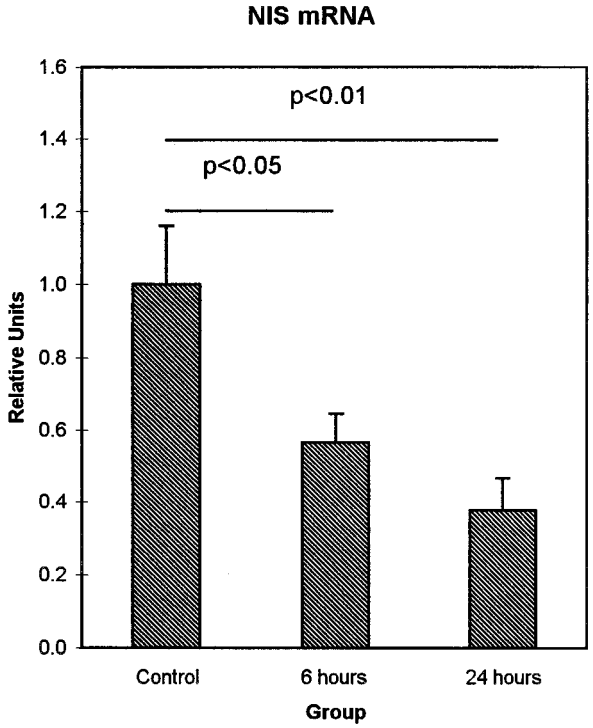

FIG. 5. Rat thyroid NIS mRNA levels, during acute iodide administration, as determined by Northern blotting. Band density was measured using a Phosphorimager. Results are pooled from two separate experiments and are normalized, with respect to individual thyroid cyclophilin mRNAs. They are expressed as relative units, with the control group mean = 1 [control group (n = 10), 6-h group (n = 10), and 24-h group (n = 9)]. Shown are the means ± SEM of each group. A P value of <0.05 is significant.

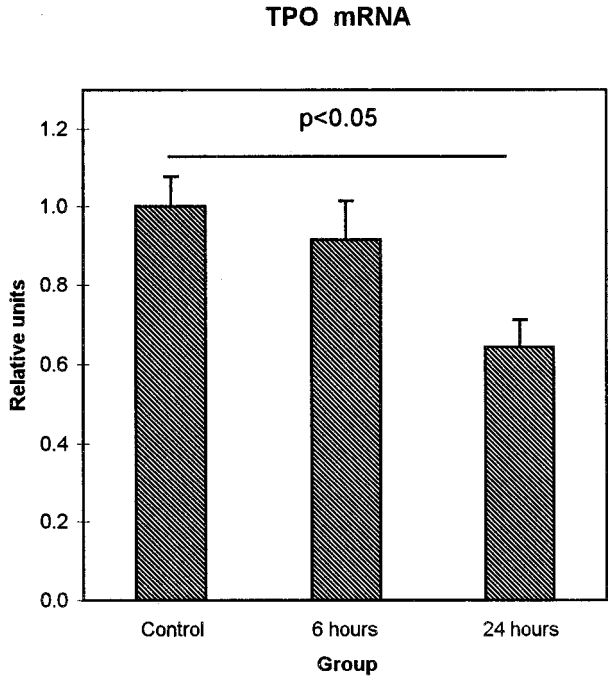

FIG. 6. Rat thyroid TPO mRNA levels, during acute iodide administration, as determined by Northern blotting. Band density was measured using a Phosphorimager. Results are pooled from two separate experiments and are normalized, with respect to individual thyroid cyclophilin mRNAs. They are expressed as relative units, with the control group mean = 1 [control group (n = 10), 6-h group (n = 10), and 24-h group (n = 9)]. Shown are the means ± SEM of each group. A P value of <0.05 is significant.

the thyroid (9, 10). Of note, the serum TSH concentrations increased, but not significantly, in response to the decreased serum T_4 and T_3 values after 1 day of iodide administration. It is possible that a greater rise occurred during the night, before the midmorning termination of the chronic iodide experiment.

It had been postulated in 1963 that the adaptation to or escape from the acute Wolff-Chaikoff effect was caused by a decrease in the active transport of iodide from the plasma into the thyroid, thereby decreasing the high concentrations of intrathyroidal iodide that inhibit hormone synthesis. We have now reinvestigated this postulate by determining the level of NIS mRNA and protein in the thyroids of iodide-exposed rats. The level of NIS mRNA significantly decreased after 1 and 6 days of iodide ingestion. The decrease in NIS protein was even more dramatic, decreasing to approximately 23% of the control values at both time points. The decrease in NIS occurred in the absence of a significant increase in TSH, which would have increased NIS or reduced the iodide induced-decrease in NIS, because TSH has been shown to increase NIS expression (11, 12). The decrease in NIS expression, after 1 day of excess iodide exposure, suggested the possibility that the active transport of iodide into the thyroid, which induces the acute Wolff-Chaikoff effect, might occur rapidly (<24 h) after iodide administration. To evaluate this hypothesis, rats were killed from 1–24 h after the acute administration of excess iodide ip. No change in NIS mRNA occurred at 1 or 2 h after iodide administration, but a significant decrease was observed at 6 and 24 h. Also, no change in NIS protein was observed at 6 h, when NIS mRNA was already decreased; however, a marked decrease in NIS protein was found at 24 h. These findings suggest that the decrease in active iodide transport into the thyroid occurred between 6 and 24 h after excess iodide exposure. Thus, it is likely that the excess iodide transported into the thyroid, before the decrease in NIS protein, was responsible for the acute inhibition of hormone synthesis previously described (2, 13). Uyttersprot *et al.* (14) recently reported that the acute administration of a single small dose of iodide to the iodine-deficient, propylthiouracil-, and perchlorate-treated dog, with elevated serum TSH concentrations, decreased NIS mRNA in the thyroid at 48 h. They did not measure NIS protein. Also, they did not determine serum iodine concentrations. Our observations are also likely more physiological, because the rats were on a normal iodine intake, were not receiving any antithyroid drugs, and did not have markedly elevated serum TSH values.

The present results also suggest that the half-life of NIS protein in the thyroid, in the presence of excess iodine, is less than 24 h, which is far shorter than the half-life of 4 days, observed in FRTL5 cells *in vitro* (15, 16). It is possible that the degradation of NIS protein is higher in the presence of excess iodide, and further studies of the half-life of NIS protein, in the presence and absence of excess iodide, are indicated. Because both the NIS mRNA and NIS protein are decreased, it seems likely that NIS regulation by iodine is (at least partly)

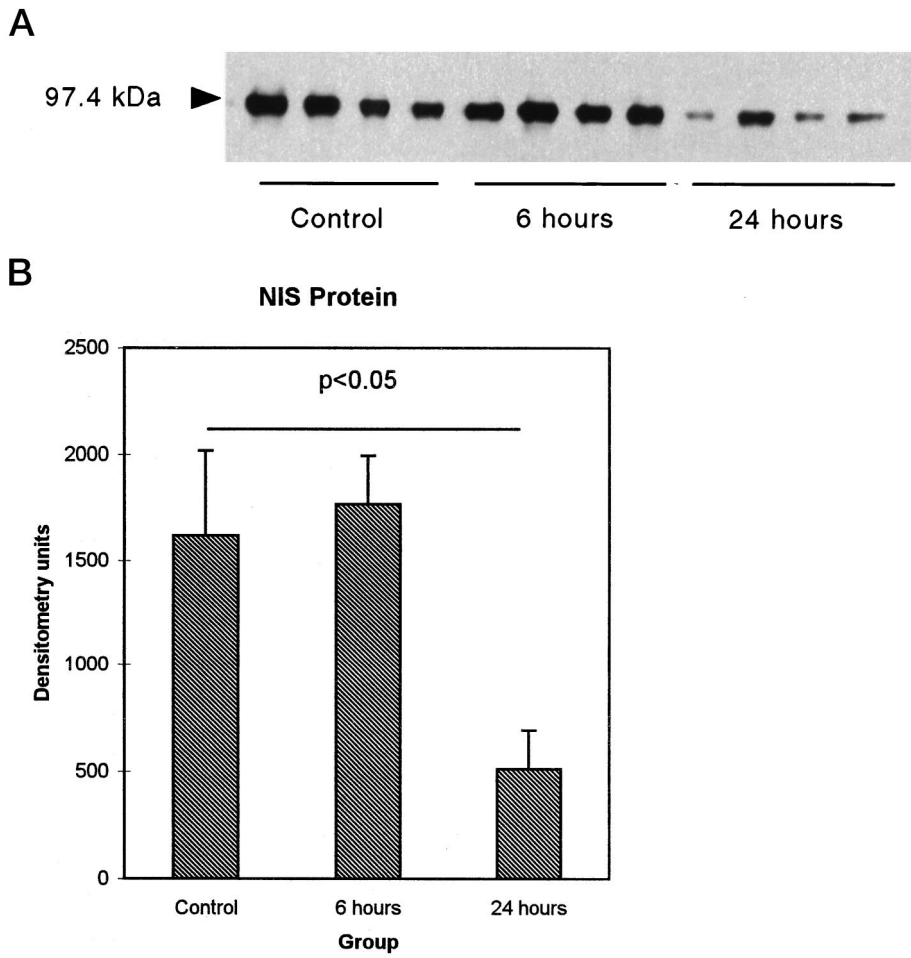

FIG. 7. Western blot analysis of NIS protein in thyroid tissue: acute excess iodide. A, Autoradiograph of Western blot of thyroid protein, extracted from rats subjected to acute excess iodide. The blot was hybridized with a primary anti NIS antibody and a secondary horseradish peroxidase-conjugated antibody. Protein detection was by enhanced chemiluminesence. Lanes 1–4 are controls, lanes 5–8 are from rats in the 6-h group, and lanes 9–12 are from rats in the 24-h group. The NIS protein is approximately 90 kDa. B, Rat thyroid NIS protein levels, during acute iodide administration, as determined by Western blot analysis. Band density was measured using a densitometer. Results are from 1 experiment and are expressed as densitometry units [control group (n = 4), 6-h group (n = 4), and 24-h group (n = 4)]. Shown are the means ± SEM of each group. A P value of <0.05 is significant.

transcriptional, although a posttranscriptional mechanism cannot be ruled out. Pulse-chase experiments are planned to examine the translational or posttranslational effects of iodine on NIS protein turnover.

Although excess iodide administration did not affect Tg or TSHr mRNAs, TPO mRNA levels were decreased after 6 days of chronic iodide ingestion and 1 day after the acute administration of a large dose of iodide. These findings are similar to those observed in the iodine-deficient hypothyroid dog, 1 day after the administration of an acute dose of iodine (14). The decrease in TPO mRNA, after iodine administration, would tend to negate the escape phenomenon. Indeed, if the decrease in TPO mRNA occurred alone, organification of iodine and subsequent hormone synthesis would continue to be impaired. In spite of this possible decrease in TPO, the reduction in iodide transport and subsequent decrease in thyroidal iodine content would permit iodine organification and hormone synthesis to resume.

We have recently reported that chronic iodine administration to the diabetes and lymphocytic thyroiditis (LT) prone BB/Wor rat increases the incidence of LT and decreases TPO mRNA in the follicular cells in contact with infiltrating lymphocytes (17). Although spontaneous LT or iodine-induced LT does not occur in iodine-sufficient or -deficient Sprague Dawley rats (18), it is possible that a decrease in TPO mRNA might be one of the mechanisms responsible for iodine-induced hypothyroidism, so common in Hashimoto's thyroiditis (19).

In summary, we have shown that excess iodide, given to rats, chronically or acutely decreases both thyroid NIS mRNA and protein. Our findings are consistent with the hypothesis that the escape from the Wolff-Chaikoff effect is caused by a down-regulation of the NIS, resulting in decreased iodide transport into the thyroid. This would then lower the intrathyroidal iodine below a critical threshold and would allow organification to resume. The decrease in NIS is likely to be, at least in part, transcriptional. In addition, we have also found that excess iodide decreases TPO mRNA and that this decrease may contribute to iodide-induced hypothyroidism commonly seen in patients with Hashimotos's thyroiditis.

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