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Uptake and Gene Expression with Antitumoral Doses of Iodine in Thyroid and Mammary Gland: Evidence That Chronic Administration Has No Harmful Effects

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Several studies have demonstrated that moderately high concentrations of molecular iodine (I_2) diminish the symptoms of mammary fibrosis in women, reduce the occurrence of mammary cancer induced chemically in rats (50–70%), and have a clear antiproliferative and apoptotic effect in the human tumoral mammary cell line MCF-7. Nevertheless, the importance of these effects has been underestimated, in part because of the notion that exposure to excess iodine represents a potential risk to thyroid physiology. In the present work we demonstrate that uptake and metabolism of iodine differ in an organ-specific manner and also depend on the chemical form of the iodine ingested (potassium iodide vs. I_2). Further, we show that a moderately high I_2 supplement (0.05%) causes some of the characteristics of the "acute Wolff-Chaikoff effect"; namely, it lowers expression of the sodium/iodide symporter, pendrin, thyroperoxidase (TPO), and deiodinase type 1 in thyroid gland without diminishing circulating levels of thyroid hormone. Finally, we confirm that I_2 metabolism is independent of TPO, and we demonstrate that, at the doses used here, which are potentially useful to treat mammary tumors, chronic I_2 supplement is not accompanied by any harmful secondary effects on the thyroid or general physiology. Thus, we suggest that I_2 could be considered for use in clinical trials of breast cancer therapies.

Introduction

Most investigations of iodine metabolism in humans and animals have focused on its role in thyroid function. Public health policies have been established to supply deficient populations with the necessary amount of this element and eradicate the iodine deficiency diseases, primarily cretinism (1). However, considerable evidence indicates that iodine *per se* could also be implicated in the physiology of other organs, such as the mammary gland (2,3). In humans the total amount of iodine in the body is 30–50 mg, and less than 30% of it is present in the thyroid gland and its hormones. About 60% of total iodine is nonhormonal and is concentrated in extrathyroidal tissues, where its biological role is still unknown (4). Moreover, it has been demonstrated that iodine distribution in the organism depends on the chemical form of iodine ingested, and that molecular iodine (I_2) is not totally reduced to iodide (I^-) in the blood before it is absorbed systemically from the gastrointestinal tract (5). Indeed, in iodine deficiency conditions, I^- appears to be more efficient than I_2 in restoring the thyroid gland to normal from a goitrous state, whereas I_2 is distinctly more effective in diminishing mammary dysplasia and atypia resulting from iodine deficiency (6). In euthyroid conditions, I_2 supplementation diminishes the symptoms of mammary fibrosis in women (7,8), causes a 50–70% reduction in the occurrence of mammary cancer induced chemically in rats (9,10), and has a clear antiproliferative and apoptotic effect in the human tumoral mammary cell line MCF-7 (11,12). Nevertheless, these findings have been viewed with caution because exposure to moderate or high iodine is thought to be a potential risk to thyroid physiology (leading to hypothyroidism or autoimmune disease) as well as to general health (retinal damage). Careful examination shows that pathological responses occurred at low or moderate iodine intake in patients with underlying or evident thyroid pathology (e.g., Hashimoto's thyroiditis, history of treated Graves' diseases, etc.) or in normal subjects only with excessively high doses of iodine or I^- (>20 mg/day; reviewed in Refs. 13–15). In contrast, no damaging effects were reported in either the human or animal studies (6–10) that used therapeutic I_2 concentrations (3–15 mg/day).

The lack of damage by moderate iodine excess might be explained by mechanisms called "thyroid autoregulation" that were first described in 1940 by Morton, who observed that moderate amounts of I^- inhibited the formation of thyroid hormones (THs) by incubated sheep thyroid slices (16).

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In fact, the thyroid gland has regulatory mechanisms that maintain normal synthesis and secretion of TH over a wide range of iodine availability. Currently, the recommended daily iodine intake is 150 μg/day, and the safe upper limit (UL) is 1.1 mg/day which comes from the Braverman group's studies in humans, in which more than 1.5 mg of I^- daily induced small but significant decreases in serum thyroxine (T4) and triiodothyronine (T3) concentrations and a significant increase in the thyrotropin (thyroid-stimulating hormone, TSH) concentration (17). When moderately high amounts (3–10 times the UL) of I^- are given to euthyroid subjects, a transient decrease in the synthesis of TH occurs for 24–48 hours. This inhibition of TH synthesis is called "the acute Wolff-Chaikoff effect," and it is due to increased intrathyroid iodine concentrations, which inhibit the iodination of tyrosyl residues of thyroglobulin (Tg) by thyroperoxidase (TPO). After 48 hours of I^- excess, the thyroid gland escapes from this effect by an adaptation that decreases the thyroid iodine trap, thereby decreasing the intrathyroid iodine concentration (18). Excess I^- inhibits the expression of the mRNA and protein of the sodium/iodide symporter (NIS) and other thyroid functions, as well as TPO mRNA expression, Tg proteolysis, hormone secretion, thyroid blood flow, glucose and amino acid transport, and thyroid growth (19–21). Two different mechanisms have been proposed to explain these effects. The first is that iodine inhibits adenylate cyclase activity, reducing accumulation of cAMP (22), and the second is that excess iodine inhibits hydrogen peroxide (H_2O_2) generation, which is crucial for TH synthesis catalyzed by TPO (23). The Wolff-Chaikoff effect is prevented by inhibitors of I^- organification, such as methimazole (MMI) and propylthiouracil (PTU); therefore, the existence of a putative organic iodine compound that mediates this effect has been proposed (21).

Another possibility to explain the lack of deleterious effects on thyroid physiology when therapeutic I_2 treatments have been applied against mammary tumors is that the mechanisms for uptake and metabolism of I_2 might be different from those well established for I^-. Our group has recently shown in several mammary tumor cell lines that I_2 is taken up by a facilitated diffusion mechanism that is NIS and pendrin (PEN) independent (11). PEN is another iodine transporter present in thyroid and mammary gland epithelium. Although in normal thyroid it catalyzes apical I^- transport, it could also participate in the influx of iodine when the cell losses its polarity, that is, in tumoral tissues (24–26). Studies of the uptake and metabolism of I_2 in thyroid or other tissues *in vivo* do not exist. Thus, the present study was designed to (i) analyze the uptake of I_2 in thyroid and mammary gland under different physiological and pathological conditions and (ii) evaluate the effects of therapeutic concentrations of this chemical form of iodine in both tissues. Our data show that (i) I_2 uptake is lower than I^- uptake, and it is partially dependent on NIS in thyroid and lactating mammary gland; (ii) in nubile mammary gland, either normal or tumoral, the uptake of I_2 is independent of NIS and PEN; (iii) a moderately high concentration of I_2 triggers the Wolff-Chaikoff effect in thyroid gland, that is, diminished NIS, TPO, and deiodinase type 1 (D1) expression, but without the typical transient decrease in the levels of circulating THs; (iv) in I_2-supplemented animals, the patterns of gene expression and circulating hormones are not changed when TPO activity is blocked with MMI; and (v) chronic (3 weeks) treatment with 0.05% I_2 increases expression of NIS, PEN, and lactoperoxidase (LPO) in tumoral mammary tissue without any alteration in thyroid physiology. These data indicate that the uptake and metabolism of iodine are organ-specific and differ depending on the chemical form in which it is ingested, and they provide additional evidence that a chronic, moderately high I_2 supplement causes no harmful secondary effects on health (e.g., body weight, thyroid economy, or reproductive cycle). Thus, we propose that I_2 supplementation should be considered for use in clinical trials of breast cancer therapies.

Materials and Methods

Animals

Female Sprague-Dawley rats were obtained from the vivarium of the Instituto de Neurobiología, UNAM-Juriquilla. Rats were housed in a temperature-controlled room ($21 \pm 1^\circ$C) with a 12-h light/dark schedule. They were given food (Purina rat chow; Ralston Purina, St. Louis, MO) and water *ad libitum*. All of the procedures followed the Guidelines of the UNAM Animal Care and Use Committee.

Carcinogen treatment

At 7 weeks of age, one group of rats were anesthetized with an aqueous solution containing 30 mg ketamine and 6 mg xylazine (Aveco, Fort Dodge, IA) per kg body wt. and treated with a single intraperitoneal (i.p.) injection of 50 mg/kg body wt. of methylnitrosourea (MNU; Sigma, St. Louis, MO). MNU was dissolved in 0.9% saline, pH 5.0, and activated by heating to 50–60°C (27). After 2 months tumors appeared, and experiments were carried out when they reached 1 cm in diameter.

Radioiodide and radioiodine uptake assay

Oxidation of $^{125}I^-$ to $^{125}I_2$ was achieved by reacting $Na^{125}I$ (NEN Life Science Products, Boston, MA) with H_2O_2 and HCl according to the method described by Thrall *et al*. (5), in which the oxidation of I^- to I_2 is 100%. Normal female rats and MNU-tumor rats received i.p. doses of 50 μCi of either $^{125}I^-$ or $^{125}I_2$. In a parallel group of rats, 6 mg of perchlorate (ClO_4^-, a specific inhibitor of NIS), 3 mg of furosamide (F, inhibitor of PEN), or both were administered i.p. 2 hours before ^{125}I injection. All animals were sacrificed 1 hour after ^{125}I administration. Thyroid, inguinal mammary glands, mammary tumoral tissue, liver, and blood were collected, and their radiolabel was measured in a γ-counter (Packard, Palo Alto, CA). Only animals with similar blood radiolabel readings (differences $< 5\%$) were included. To adjust for possible differences in dose of radioactivity, the data were normalized as radioactivity uptake compared to liver (non-uptake organ), by the following formula: (cpm/mg for thyroid, mammary gland, or tumor)/(cpm/mg for liver).

Wolff-Chaikoff effect test

The Wolff-Chaikoff effect was analyzed in thyroid, mammary glands, and tumors from nubile rats at 1 and 6 days after potassium iodide (KI) or I_2 administration. The rats were divided into four groups and given access *ad libitum* to distilled water containing: no addition (control), 0.05% KI (I^-), or 0.05% I_2. After either 1 or 6 days of iodine treatment, animals were

sacrificed, and the thyroid, mammary glands, and liver from each rat were removed, immediately frozen, and stored at −70°C for measurement of mRNA expression and/or D1 activity. Serum was collected and frozen for later measurement of iodine, THs (T4 and T3), and TSH. In the second group we tested whether TPO was involved in establishing the Wolff-Chaikoff effect. From 2 days before the treatment with KI or I_2 and to the end of the study, these animals were given drinking water supplemented with 0.05% MMI.

Chronic treatment with I_2 or I^- of rats with or without mammary cancer

Animals with mammary tumors were given 0.05% KI or I_2 in their drinking water for 3 weeks. Normal, age-matched animals were used as control group. Thyroid and mammary glands were dissected, and levels of gene expression, circulating hormone, and iodine were analyzed.

Quantitative real-time PCR

Two micrograms of total RNA extracted from thyroid or mammary gland (TRIzol reagent; Life Technologies, Carlsbad, CA) was reversed transcribed (Superscript II system; Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. PCR was performed on sequence detector system Roto-Gene 3000 (Corbett Research, Mortlake, NSW, Australia) using SYBR green as a marker for DNA amplification. The reaction was performed with 1 μL cDNA template and the quantitative real-time PCR (qPCR) supermix-UDG kit (Invitrogen), using 40 cycles of three-step amplification (94°C for 30 s, 55–58°C for 30 s, 72°C for 30 s) and the gene-specific primers listed in Table 1. PCR generated only the expected specific amplicon, which was demonstrated in each case by the melting temperature profile (dissociation curve) and by electrophoresis of 5 μL of the PCR product through a 2% agarose gel containing ethidium bromide in TAE (0.04 M Tris-Acetate/0.01 M EDTA electrophoresis buffer). No PCR products were observed in the absence of template. Gene expression was calculated using the Δcycle threshold (Δct) method and normalized to content of cyclophilin, a nonregulated housekeeping gene (28). All measurements were performed in triplicate.

D1 enzyme assay

D1 activity was determined by a modification of the iodine-release method standardized for liver and mammary gland (29). Tissues were homogenized in 10 mM HEPES (pH 7.0) containing 0.32 M sucrose, 1.0 mM EDTA, and 10 mM DTT, and centrifuged at 2800 *g* for 30 min at 4°C. Assay conditions were as follows: 2 μg protein for liver or 200 μg for mammary gland, 2 nM radiolabeled reverse triiodothyroine [^{125}I]rT3, 0.5 μM unlabeled rT3, and 5 mM DTT. After a 1- or 3-hour incubation, released acid-soluble ^{125}I was isolated by chromatography on Dowex 50W-X2 columns. Proteins were measured by the Bradford method (Bio-RAD protein assay; Bio-Rad Laboratories, Richmond, CA). Results are expressed as pico- or micromoles of iodine released per mg protein per hour [I/(mg protein hour)] for thyroid or mammary tissue, respectively.

Hormone and iodine concentrations

Serum T4 and T3 were measured in duplicate by the homologous radio immuno assay (RIA) method (30). Serum TSH was analyzed by the Biotrack assay system (rat RIA method; Amersham, Piscataway, NJ), and free I^- was measured by ion chromatography (31,32) with a Dionex ion chromatography system, model (DX-500) equipped with a Dionex pulsed amperometric detector, ion Pac AG11 and As11 columns, and a silver working electrode. The mobile phase was 50 mM nitric acid at room temperature and a flow rate 1.0 mL/min. As internal standard curve, 10–3000 μg/dL of KI was used. Serum samples (10 μL) diluted in distilled water (1:100) were analyzed (33).

Statistical analysis

The data are expressed as mean ± SD. Differences between experimental groups were analyzed using one-way ANOVA and Tukey's honest significant difference test. Differences with $p < 0.05$ were considered statistically significant.

Results

Radioiodide and radioiodine uptake assay

Thyroid and mammary glands (lactating, nubile, or tumoral) were assayed for the uptake of radioactive I^- and I_2. All results were normalized to the uptake observed in liver, which does not accumulate iodine. Figure 1 illustrates that thyroid and mammary glands can accumulate both types of iodine and shows that thyroid and lactating mammary gland exhibit a significant uptake of I^-, which is internalized by NIS in mammary gland and by both NIS (inhibition with ClO_4^-) and PEN (inhibition with furosemide, F) in thyroid. The data also show that in these tissues, I_2 uptake is three times less than I^- uptake, and only about half of this I_2 capture is inhibited by ClO_4^-. In contrast, in nubile animals, normal or tumoral mammary tissue captured 300 times less iodine than thyroid and 4 times less than lactating mammary

Table 1. Oligonucleotide Sequences

Gene	*Sense sequence*	*Antisense sequence*	*Size (bp)*	*Alignment temp. (°C)*
Cyc	AGA CGC CGC TGT CTC TTT TCG	CCA CAC AGT CGG AGA TGG TGA TC	519	56
NIS	CCG GAT CAA CCT GAT GGA CT	CCT GAG GGT GCC ACT GTA AG	377	58
PEN	CAT TCT GGG GCT GGA CCT C	CCT TCG GGA CAT TCA CTT TCA	487	60
LPO	AAA GCC CAG TGT GAC GAG CA	GCC GTC CAT GGT CTG AGA CT	297	55
TPO	GCT GCC TCC TGT CTA CGA AG	ATC AAG GAA GGA GGT CAA GCC	220	55
D1	CTT GGA GGT GGC TAC GG	CTG GCT GCT CTG GTT CTG	561	55

Cyc: cyclophilin; NIS: sodium/iodide symporter; PEN: pendrin; LPO: lactoperoxidase; TPO: thyroperoxidase; D1: deiodinase type 1; size: in base pairs.

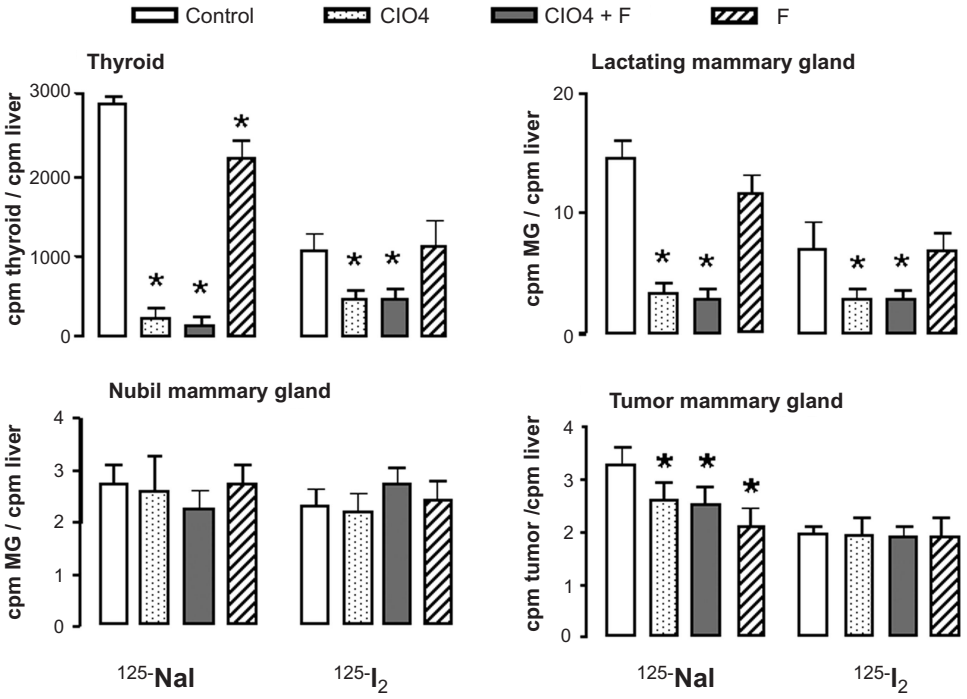

FIG. 1. ^{125}I in thyroid and mammary glands of lactating (10 days postdelivery) nubile and tumoral female rats injected with 50 μCi of either $^{125}I^-$ or $^{125}I_2$. Two hours before ^{125}I injection rats received an intraperitoneal injection of saline or 6 mg of perchlorate (ClO_4^-) or 3 mg of furosamide (F) or both ClO_4^- and F in 200 μL of saline. The rats were sacrificed 1 hour after ^{125}I administration. The data were normalized as radioactivity uptake compared to liver (nonuptake organ), and are expressed as mean $\pm$ SD ($n = 3$–6). Differences between experimental groups were analyzed using a one-way ANOVA and Tukey-HSD test. Asterisk indicates significant difference from the appropriate control ($p < 0.05$).

gland. In normal mammary gland, both types of iodine are taken up to the same extent, and neither NIS nor PEN participated in their internalization. Tumoral tissue internalizes I^- partially via NIS and PEN, and 30% more I^- is taken up than I_2; I_2 uptake is independent of NIS or PEN.

Wolff-Chaikoff effect

Figure 2 shows thyroid NIS, PEN, TPO, and D1 expression after 1 or 6 days of I^- or I_2 supplements in nubile animals. Our data show that thyroid gland exhibited saturation in the uptake of I^- and that the mechanism to adapt to excess iodine involved the downregulation of components implicated in the uptake (NIS and PEN), organification (TPO), and metabolism (D1) of iodine and THs. This mechanism, known as the acute Wolff-Chaikoff effect, was observed with both chemical forms of iodine and remained after 6 days for NIS and PEN expression; TPO expression stayed low with I_2. In contrast, I^- lowered D1 and TPO expression on day 1, but these reductions were not significant on day 6. The figure also shows the expression of these thyroid genes when organification of I^- is inhibited by MMI, which blocks the synthesis and activity of the enzyme TPO. The data show that in the presence of MMI, only I_2 inhibits NIS and PEN expression, indicating that the ability of I^- to cause this inhibition requires its oxidation via TPO.

Figure 3 shows the expression of mammary NIS, PEN, LPO, and D1 in nubile animals after 1 or 6 days of I^- or I_2 supplements, with and without MMI. PEN expression increases in mammary gland on the sixth day of I_2 supplement, with or without MMI. To confirm organ-specific responses, we analyzed the effect of iodine excess on D1 activity in thyroid and mammary glands of these same animals. As shown in Figure 4, the iodine treatment caused a transient decrease in thyroid D1 activity on day 1 but had no effect on D1 activity in mammary tissue. This pattern is similar to that observed for expression of D1 mRNA (see Figs. 2 and 3).

Figure 5 shows the serum concentrations of I^-, THs, and TSH in the animals described above. Measurement of I^- confirmed that the iodine-supplemented animals had high serum I^- concentrations. These results also confirm that excess I^- is accompanied by a transient decrease in serum T4 and T3 levels on day 1. The decrease in circulating levels of T4 and T3, as well the increase in TSH in control and I^--supplemented animals treated with MMI, indicates the involvement of TPO in TH synthesis. In contrast, and even though I_2 supplementation is accompanied by a decrease in thyroid NIS, PEN, TPO, and D1 expression, the serum concentrations of THs were not reduced by I_2 treatment. The possibility that I_2 could be incorporated into Tg and thereby generate THs independent of TPO action is supported by results obtained in MMI animals, whose basal concentrations of T4, T3, and TSH were normal.

Figure 6 summarizes the changes in NIS, PEN, TPO, and D1 expression in thyroid gland from animals with and without mammary tumors, chronically exposed to 0.05% I_2 in their drinking water. We used this dose previously in a chronic treatment and found that it significantly reduced the incidence of mammary tumors induced by MNU (10). The present data show that after 3 weeks of the I_2 supplement,

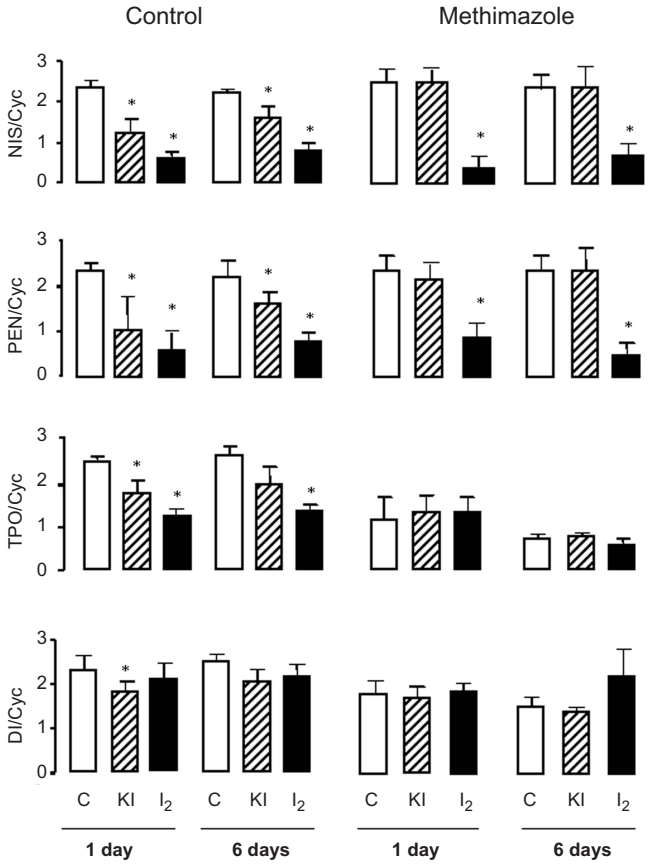

FIG. 2. Effect of iodine excess on gene expression in thyroid gland. Drinking water contained distilled water only (control; C) or was supplemented with 0.05% potassium iodide (KI) or 0.05% iodine (I_2) for 1 or 6 days. To block thyroperoxidase (TPO) activity, 0.05% methimazole was added to the drinking water 2 days before the iodine supplement. Expression of the mRNAs for sodium/iodide symporter (NIS), pendrin (PEN), TPO, and deiodinase type 1 (D1) was measured by the real-time PCR method. Cyclophilin (Cyc) served as an internal control and was used to normalize for differences in input RNA. Data are expressed as mean ± SD ($n = 3$). Differences between experimental groups were analyzed using a one-way ANOVA and Tukey-HSD Test. Asterisk indicates significant difference from the appropriate control ($p < 0.05$).

the lower expression of thyroid NIS and PEN mRNA is maintained in both groups. Figure 7 shows the NIS, PEN, LPO, and D1 expression in normal or tumoral mammary gland of these same groups. Normal and tumoral tissues exhibit differential responses to I_2: in normal mammary tissue, I_2 has no effect, whereas in tumoral mammary gland it increases NIS, PEN, and LPO expression. In spite of the changes observed in thyroid or tumoral mammary tissue, circulating levels of TH and TSH remain constant in all I_2-treated groups. High levels of serum I^- confirm the elevated iodine consumption by these animals (Fig. 8).

Discussion

Exposure of the thyroid gland to high levels of I^- results in the acute Wolff-Chaikoff effect, in which TH synthesis is inhibited. This inhibition is transient, lasting from 24 to 48 hours, after which the thyroid has adapted to prolonged iodine excess, resuming near-normal hormone synthesis (18). In the present experiments, we compared the effects on uptake and gene expression in thyroid and mammary glands from animals exposed to moderately high levels of iodine using two different chemical forms: I^- and I_2. In terms of uptake, our results show that, although both types of iodine were internalized by both glands, I^- was predominantly taken up by thyroid; assimilation was much lower in the mammary gland, but significantly greater in lactating than in nubile and tumoral mammary gland. In thyroid and tumoral mammary glands, NIS and PEN were involved in I^- internalization; in lactating mammary gland, only NIS participated, and in nubile mammary gland neither of these transporters were involved. In contrast, I_2 uptake was partially inhibited by ClO_4^- in thyroid and lactating mammary gland, but not in nubile or tumoral mammary gland, suggesting that I_2 is primarily internalized by components other than NIS or PEN. Moreover, it is possible that the partial inhibition of I_2 uptake by ClO_4^- observed in thyroid and lactating mammary gland might actually correspond to

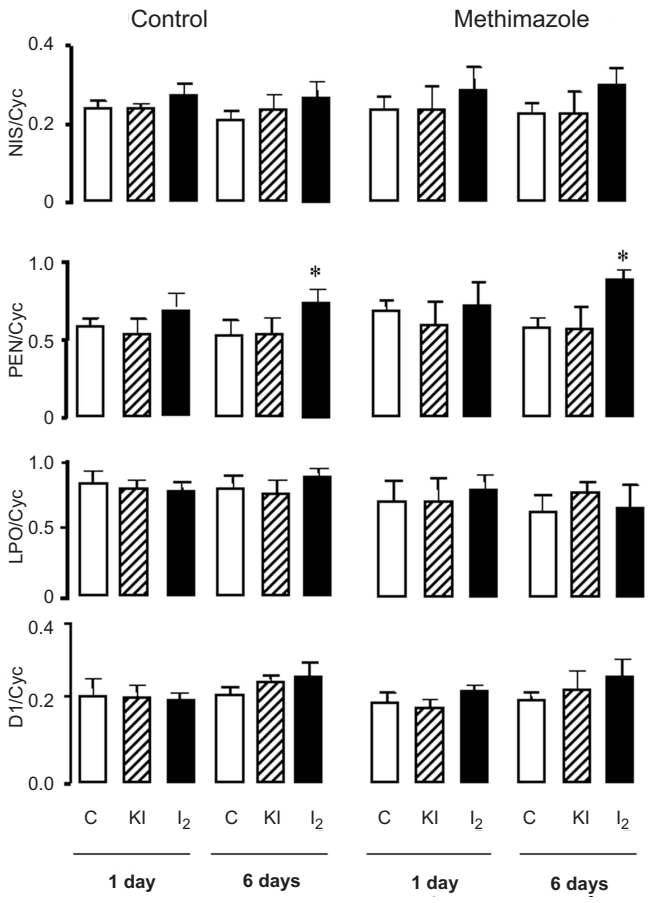

FIG. 3. Effect of iodine excess on gene expression in mammary gland. All treatments and measurements were the same as in Figure 2 except that mRNA expression for lactoperoxidase (LPO) rather than thyroperoxidase (TPO) was measured. Asterisk indicates significant difference from the appropriate control ($p < 0.05$).

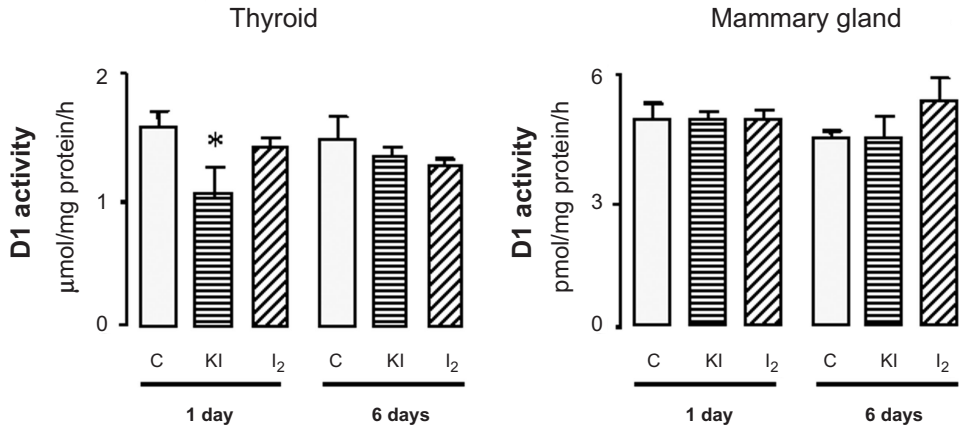

FIG. 4. Deiodinase type 1 activity in thyroid and mammary gland from nubile animals. Control and iodine supplements were as in Figures 2 and 3, and the results are expressed as mean ± SD ($n = 4$). Asterisk indicates significant difference from the appropriate control ($p < 0.05$).

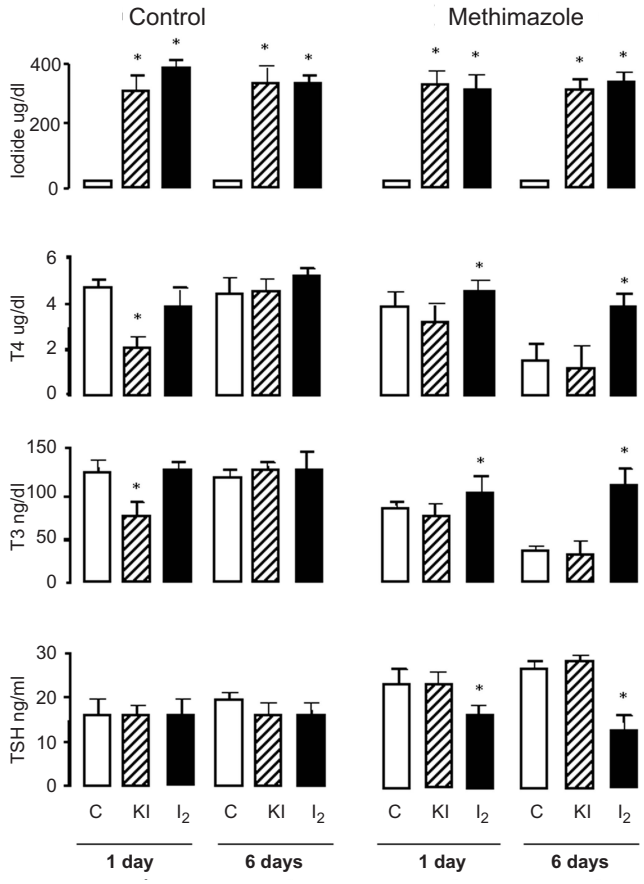

FIG. 5. Circulating levels of iodide and thyroid hormones in nubile animals. Treatments were as in previous figures. Iodide was measured by high performance liquid chromatography, thyroxine (T4), triiodothyronine (T3), and thyrotropin (TSH) by radio immuno assay method. Data are expressed as mean ± SD ($n = 6$). Asterisk indicates significant difference from the appropriate control ($p < 0.05$).

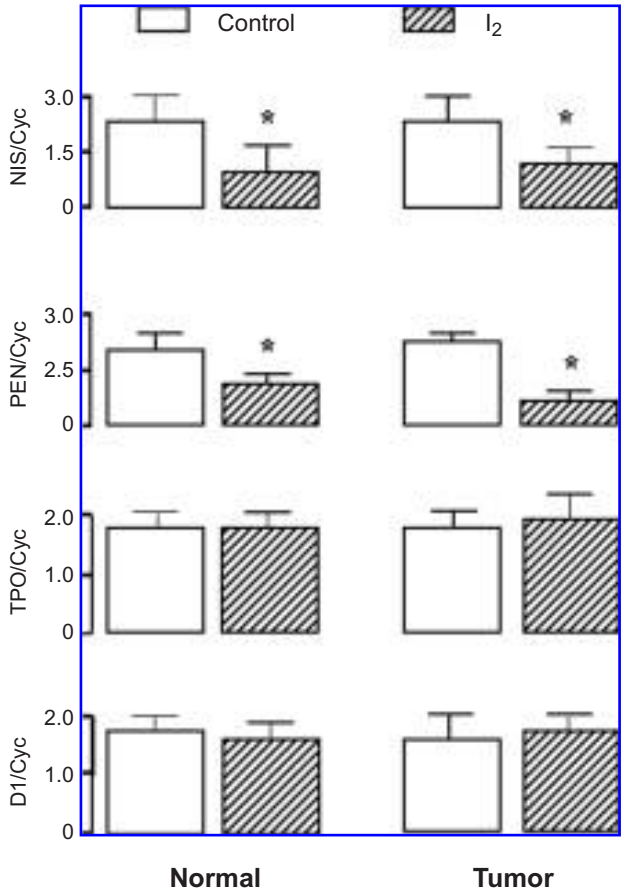

FIG. 6. Effect of chronic iodine supplement on gene expression in thyroid gland from normal or mammary tumoral rats. Drinking water contained distilled water only (control; C) or was supplemented with 0.05% iodine (I_2) for 3 weeks. Gene expression measurements were the same as in Figure 3. Asterisk indicates significant difference from the appropriate control ($p < 0.05$).

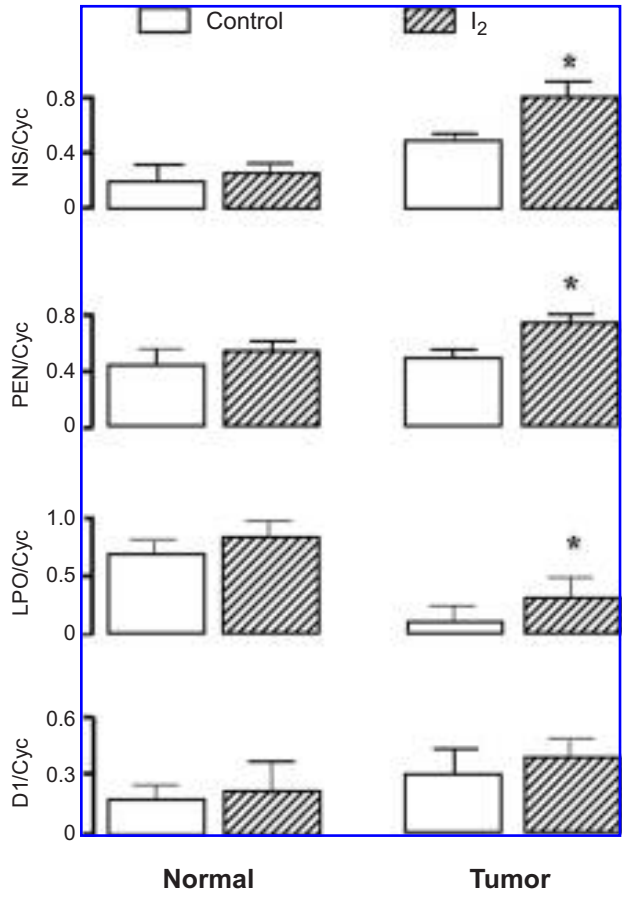

FIG. 7. Effect of chronic iodine supplement on gene expression in mammary gland from normal or mammary tumoral rats. Treatments and measurements were as in Figure 6. Asterisk indicates significant difference from the appropriate control ($p < 0.05$).

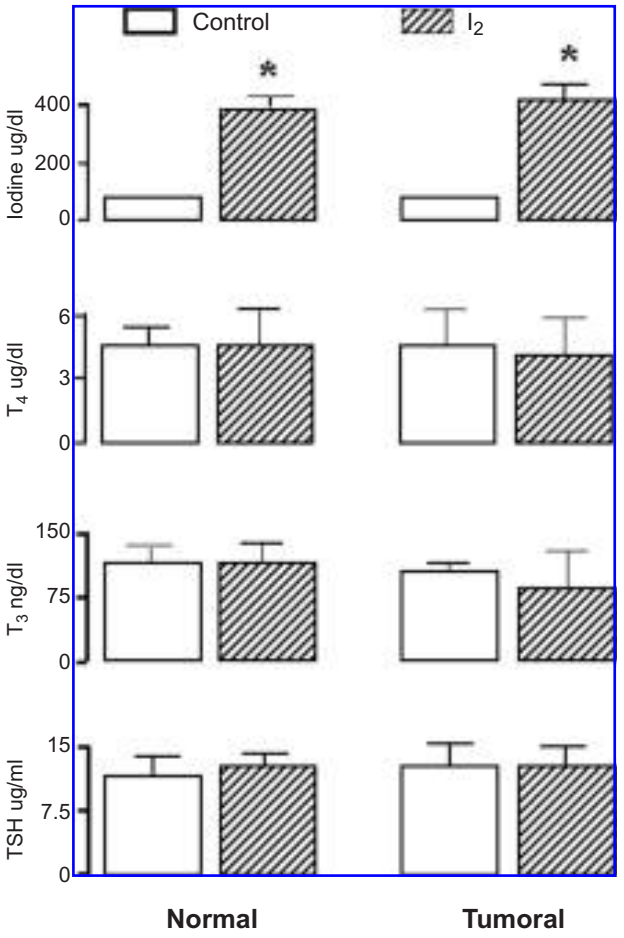

FIG. 8. Circulating levels of iodine and thyroid hormones (THs) after 3 weeks of I_2 treatment in normal or mammary tumoral rats. Iodine hormone and TH were measured as in Figure 5. Data are expressed as mean ± SD ($n = 6$). Asterisk indicates significant difference from the appropriate control ($p < 0.05$).

inhibition of the uptake of I^- that was generated in the organism by reduction of I_2. In previous studies it was suggested that when the iodine supplement is given orally, a fraction of the I_2 is reduced to I^- in the gastrointestinal tract, and some proportion of I^- is oxidized to I_2 by the peroxidase activity present in the small intestine (5,6). This hypothesis is consistent with our data showing that in normal nubile mammary gland, which expressed very low amounts of NIS, uptake of both types of iodine was similar and neither of them was inhibited by ClO_4^- or F. Second, in lactating mammary gland the residual radioactivity detected in ClO_4^- or F-treated animals was similar in magnitude to the levels exhibited in normal or tumoral tissue, which probably correspond to I_2. Third, in mammary tumors, which expressed almost twice as much NIS as normal mammary gland, ClO_4^- inhibited I^- uptake by only 15%.

Although the exact mechanism involved in the uptake of I_2 has not been elucidated, data of our laboratory in the human tumoral cell line MCF-7 showed that I_2 transport is a saturable and protein synthesis–dependent process that does not involve the well-established I^- transporters like NIS and PEN (11). Also relevant for our present results are studies in brown algae showing that I^- uptake is dependent on oxidation; that is, I^- in seawater is oxidized to I_2 or hypoiodous acid by exohaloperoxidases and then penetrates into algal cells by means of a facilitated diffusion system (34). Experiments in progress in our laboratory are designed to identify the nature of this putative I_2 transporter.

In relation to the effect of I^- excess, our data corroborated the earlier report that thyroid gland exhibits the classical inhibition of NIS and TPO expression, as well the transient decrease in T4 and T3 circulating levels (19). In addition, we found that both the mRNA and activity of thyroid D1 and the expression of PEN mRNA also decrease, suggesting that the I^- effect on the thyroid machinery includes, besides reducing I^- uptake, a decrease in the generation of TH. Moreover, the absence of an I^- effect on the expression of these genes when the animals were treated with MMI strongly supports the notion that the inhibitory effect of I^- requires its oxidation by TPO (23).

This is the first study on the effects of a moderately high I_2 supplement on thyroid physiology. I_2 exerts, as I^-, an inhibitory effect on NIS, PEN, TPO, and D1 expression, but without the transient decline in circulating TH levels. This finding indicates that I_2 can bind to organic components and thereby inhibit gene expression and also generate TH.

Moreover, the finding that I_2-treated animals supplemented with MMI preserve the thyroid gene expression pattern of the Wolff-Chaikoff effect reinforces the proposals that I_2, as an oxidized form of iodine, does not use TPO for its organification and that a putative organic iodine compound that mediates these effects is generated (20,23). In addition, the presence of constant levels of circulating thyronine suggests that the TH synthesis machinery could organify I_2 also. This organification could take place in thyroid gland through iodination of Tg, and/or as suggested previously, in the gastrointestinal tract where the I_2 reacts with metabolites of TH to resynthesize T4 and elevate its level in blood (35).

The last part of the present experiments shows that a chronic I_2 supplement creates an organ-specific response. The levels of NIS and PEN expression decrease in thyroid, but they remain unchanged in normal mammary glands. An interesting observation is that in tumoral mammary gland, I_2 increases the expression of NIS, PEN, and LPO, suggesting that its antitumoral effect involves mechanisms related to the uptake and oxidation of I^-. Although I_2 metabolism in tumoral mammary gland has not been reported, our studies in mammary MCF-7 cells show that I_2 supplement is accompanied by the generation of iodinated lipids with a migration similar to that of 6-iodolactone (6-IL), and that formation of this iodolipid is not blocked with PTU (11). In the thyroid gland, the antiproliferative and/or apoptotic effect of I^- treatment is mediated by iodinated arachidonic acid (AA) derivatives, such as 6-IL or iodohexadecanal (36–38). Iodolipid formation in mammary gland treated with I_2 has not been investigated *in vivo*. Several studies have reported elevated prostaglandin levels in breast cancer but not in normal mammary gland (39,40). Prostaglandins are produced from AA by the enzyme cyclooxygenase, indicating the presence of high levels of AA in breast tumors. It is possible that these high levels of AA and the iodolipids formed from them may explain the specific effect of I_2 only in tumoral tissues.

Finally, in chronically I_2-treated animals, there is a persistent decrease in thyroid NIS and PEN expression, but serum concentrations of TH and TSH are the same as those found in control animals. Goiter, hypothyroidism, elevated serum TSH values, and even ocular damage have been reported to result from ingestion of excess iodine in medications such as amiodarone, in by-products of iodine-containing water purification systems, in iodine-containing mouth rinses, or in the diet. However, in these reports, pathological responses occurred in normal subjects only when the I^- dose was extremely high (reviewed in Refs. 13–15). In contrast, several clinical trials demonstrated that moderately high levels of I_2 (3–6 mg/day) could remediate symptoms associated with chronic clinical mastalgia without thyroid or clinical effect (7,8). These differences in the thyroid response could be explained by the chemical form of iodine administrated and are in agreement with the present data showing that thyroid gland takes up less I_2 than I^- and that the Wolff-Chaikoff effect is not accompanied by changes in thyroid physiology. Thus, our data in mammary cancer models highlight the importance of I_2 in the health of the mammary gland and provide evidence that the uptake and metabolism of iodine is organ specific and depends on the chemical form in which it is ingested. In conclusion, although iodine supplementation needs to be accompanied by careful supervision, especially in subjects with underlying or evident thyroid pathologies, we propose that I_2 supplementation could be considered for use in clinical trials of breast cancer therapies.

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