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Hormonal Regulation of Radioiodide Uptake Activity and Na^+/I^- Symporter Expression in Mammary Glands*

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ABSTRACT

The observation that radioiodide uptake (RAIU) activity, mediated by the Na^+/I^- symporter (NIS), is significantly increased in lactating breast suggests that RAIU and NIS expression in mammary gland are modulated by hormones involved in active lactation. We showed that both the NIS expression level and RAIU in rat mammary gland are maximal during active lactation compared to those in the mammary glands of virgin and pregnant rats as well as the involuting mammary gland. In the lactating mammary gland, NIS is clustered on the basolateral membrane of alveolar cells as a lesser glycosylated form than NIS in thyroid. The RAIU of lactating mammary gland was partially inhibited by treatment with a selective oxytocin antagonist or bromocriptine, an inhibitor of PRL release. These findings suggest that RAIU and NIS expression in mammary gland are at least in part modulated by oxytocin and PRL. Indeed, we showed that NIS messenger ribonucleic acid level was increased in a dose-dependent manner by oxytocin and PRL in histocultured human breast tumors. (*J Clin Endocrinol Metab* **85:** 2936–2943, 2000)

THE IODIDE-CONCENTRATING activity of thyroid tissues has allowed the use of radioiodide/^{99m}Tc for scintigraphic imaging, as an adjunct to surgical therapy for ablation of postsurgical remnants, and as a treatment of residual, recurrent, and metastatic disease. Many studies have demonstrated that radioiodide therapy significantly increases the disease-free interval and survival of patients with residual, recurrent, and metastatic thyroid tumors that retain iodide-concentrating activity (1). For patients with thyroid cancers, iodide uptake activity in thyroid cancers is minimal unless it is intensively stimulated by TSH. Therefore, before radioiodide therapy, the patient's serum TSH level is elevated by T_4 withdrawal or exogenous TSH administration.

The thyroid is not the only tissue that shows radioiodide uptake (RAIU) activity. RAIU has been demonstrated in various extrathyroidal tissues, such as lactating mammary gland. It has been shown that breast atypia and malignancy have increased RAIU (2), and that breast cancers can be detected by radioiodide/^{99m}Tc scintigraphy (3, 4). Furthermore, one study indicates that high RAIU may prove to be the most specific biochemical characteristic of hormone-dependent breast tumors compared to hormone-independent tumors (5). The fact that RAIU is significantly increased in lactating breast tissues (6) suggests that RAIU is also subject to hormonal control in breast tissues, and that RAIU activity and Na^+/I^- symporter (NIS) expression may be stimulated by hormones involved in active lactation.

NIS is the molecule that mediates RAIU in target cells such as thyroid, salivary gland, and breast. Molecular cloning of NIS (7, 8) has made it possible to investigate hormonal control of NIS expression in breast tissues. As RAIU in breast is at its maximum when women are undergoing active lactation, hormones involved in active lactation are the most likely candidates to induce NIS expression in breast. PRL is the most important hormone for lactogenesis, and oxytocin (OT) is the essential hormone to maintain active lactation. An increased expression of PRL receptor (PRLR) has been documented in some human breast tumors and human breast cancer cell lines (9–11). Although oxytocin receptor (OTR) is expressed in myoepithelial cells in normal breast tissues, it has been reported to be expressed in human breast tumors (12–14). In this study we investigated both RAIU and NIS expression in rat mammary glands of various stages and the possible effects of PRL and OT on RAIU and NIS expression in breast tissues.

Materials and Methods

Animal model

Sprague Dawley rats were purchased from Harlan Sprague Dawley, Inc. (Indianapolis, IN), and housed in the animal vivarium facility at the Ohio State University. All animal studies were approved by the Ohio State University institutional laboratory animal care and use committee. For oxytocin antagonist treatment, lactating rats were injected ip with a selective oxytocin antagonist, des Gly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT (OTA) (15) at a dosage of 3 μg in 300 μL/injection. For bromocriptine (Br-CT) treatment (Sigma, St. Louis, MO), lactating rats were injected ip

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with Br-CT at a dosage of 0.6 mg in 300 μL/injection. For Br-CT plus OTA treatment, lactating rats were injected ip with 0.6 mg Br-CT and 3 μg OTA in 300 μL/injection. Every rat was injected twice a day for 1 week (lactating days 4.5–11.5). Control animals were injected with the same volume of saline. Each experimental group had three rats.

In vivo ^{99m}Tc scintigraphy

Rats were anesthetized with inhalation of isoflurane, and then 1.5 mCi ^{99m}Tc-pertechnetate ($^{99m}TcO_4^-$) in 0.2 mL volume were administered via tail vein injection. Rats were placed in a prone position on a γ-camera (QRS Systems, Nuclear Medicine Diagostics Systems Laboratories, Inc., San Antonio, TX) with a 140-keV high resolution collimator interfaced to a Macintosh computer-based nuclear medicine imaging system (NucLear MAC 2.9, Scientific Imaging, Littleton, CO). The images were obtained at 20 min postinjection of $^{99m}TcO_4^-$ with a 2-min acquisition time.

Radioiodide uptake assay and radioiodide organification assay

NIS functional activity in mammary gland was assessed by RAIU, the sum total of iodide influx and efflux in 1 h. $^{125}I^-$ was ip administered to rats at 0.3 μCi (in 0.2 mL phosphate-buffered saline)/g BW. Rats were sacrificed 1 h later, and tissues, including axillary and inguinal mammary glands, were collected to measure radioactivity using a γ-counter (Packard Instruments, Downers Grove, IL). The data were presented as a fold increase in radioactivity, compared to spleen, by the following formula: (counts/mg for mammary gland)/(counts/mg for spleen). Radioiodide organification assay was performed as previously described (16).

RIA for rat serum PRL (rPRL)

After drug treatment, blood was collected from the tail vein before RAIU assay, and sera were stored at −80 C before rPRL RIA. For rPRL RIA, a double antibody method was employed, using highly purified rPRL, AFP4459B, as the iodinated ligand, rabbit anti-rPRL, NIDDK S-9, AFP131581570, as the primary antibody, and rPRL AFP4459B, NIDDK rPRL RP-3 as the cold standard or reference preparation. These RIA immunoreagents are distributed by the NIDDK National Hormone and Pituitary Program, gratis, to researchers on request (consult website http://www.humc.edu/hormones for additional information).

Western blot analysis

Western blot analysis was performed as previously described (17) with some modifications. Briefly, frozen tissues were pounded in a vinyl bag, homogenized, and sonicated (20 s) in a lysis buffer (50 mmol/L Tris (pH 8.0), 150 mmol/L NaCl, 1 mmol/L ethylenediamine tetraacetate, 1% Nonidet P-40, 100 μg/mL phenylmethylsulfonylfluoride, 1 μg/mL aprotinin, and 1 μg/mL leupeptin). The lysates were centrifuged at 12,000 × *g* for 20 min at 4 C. The supernatants (100 or 200 μg/lane) were solubilized for 30 min at 37 C in the same volume of reducing sample buffer (0.125 mol/L Tris-HCl (pH 6.8), 4% SDS, 10% β-mercaptoethanol, and 20% glycerol) and subjected to 7.5% SDS-PAGE. Western blot analysis was then performed by incubating the filter with an anti-rNIS peptide polyclonal antibody pAb 716 (1:5000) generated by Dr. Bernard Rousset (18) in Tris-buffered saline with 0.1% Tween-20 containing 5% nonfat dry milk for 1.5 h at room temperature, followed by incubation with horseradish peroxidase-conjugated donkey anti-rabbit IgG (1:4000) for 1 h at room temperature. For deglycosylation of the proteins, 100 or 200 μg pro-

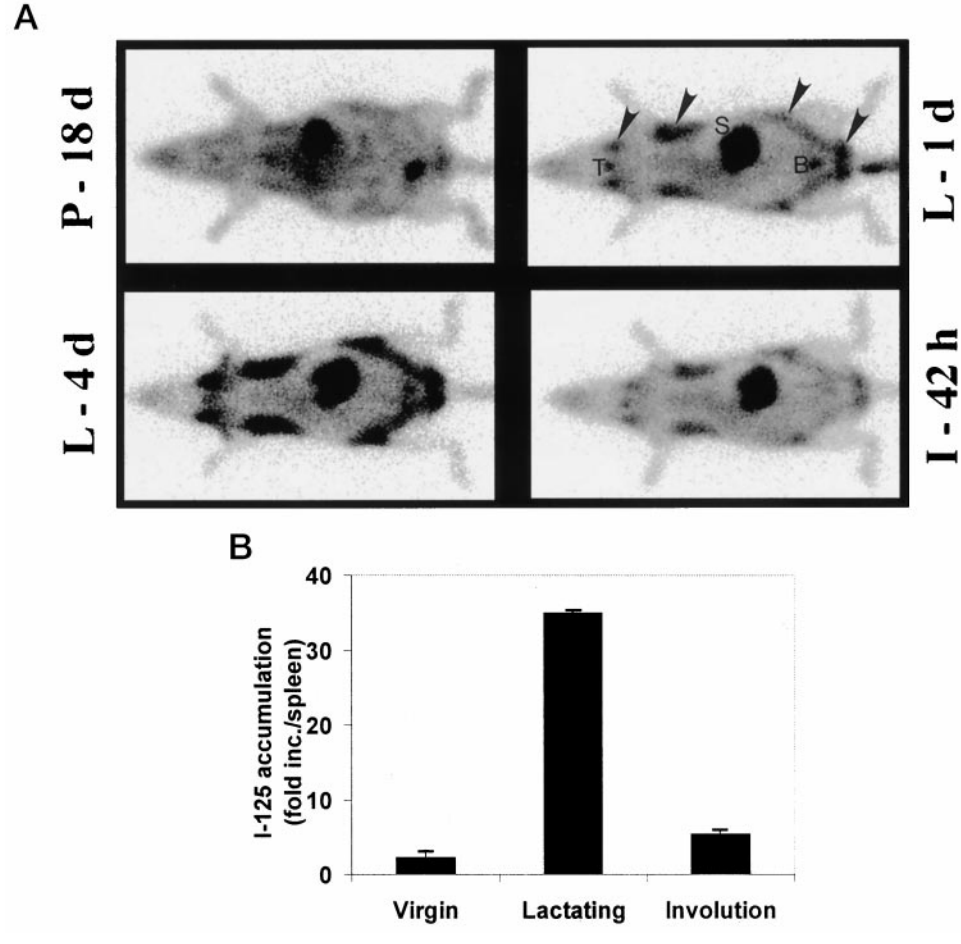


FIG. 1. A, $^{99m}TcO_4^-$ whole body scintigraphies in rats. Note that the $^{99m}TcO_4^-$ uptake activity was barely detected in the mammary glands of late pregnancy (P-18d), was evident in lactating mammary glands (L-1d, L-4d), and was dramatically decreased in the involuting mammary glands of rats that had their pups removed for 42 h (I-42h). *Arrowheads*, Mammary glands; S, stomach; B, bladder; T, thyroid. B, $^{125}I^-$ γ-counts in rat mammary glands. Rats were injected ip with $^{125}I^-$ (0.3 μCi/g BW) before death. Mammary glands of lactating rats (16 day postdelivery) had a 35 ± 0.4-fold increase in RAIU normalized to spleen RAIU, whereas mammary glands of virgin rats (11 weeks old) and mammary glands of involution had a 2.3 ± 0.9- and a 5.47 ± 0.5-fold increases in RAIU normalized to spleen RAIU, respectively. Each experimental group had two rats.

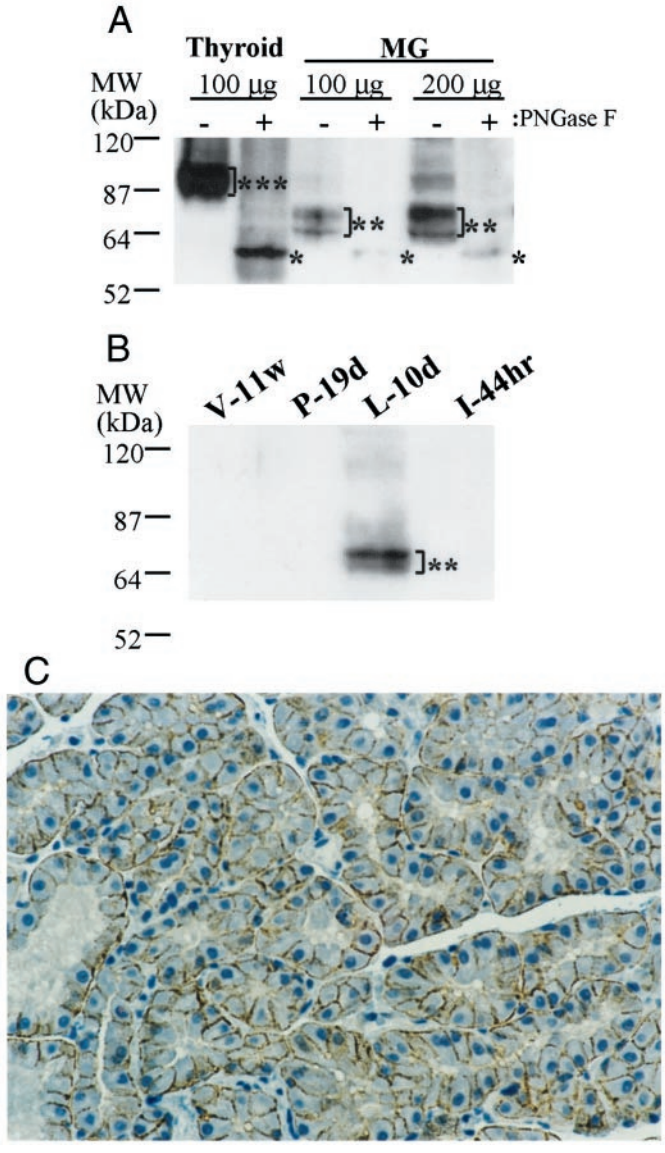


FIG. 2. A, Western blot analysis for rNIS expression in lactating mammary gland (MG) and thyroid. Note the two smaller weaker bands (**) of approximately 65 and 70 kDa in the lactating mammary gland compared to the approximately 90-kDa band (***) in thyroid. After PNGase F treatment, nonglycosylated rNIS of about 60 kDa (*) were detected in both mammary gland and thyroid. Either 100 or 200 μg cell lysates were used for Western blot analysis. B, Western blot analysis for rNIS expression in the mammary glands of 11-week-old virgin (V-11w), late pregnant (P-19d), and lactating rats at 10 days postdelivery (L-10d) as well as in the involuting mammary glands of rats that had their pups removed for 44 h (I-44h). The same amount of cell lysates (200 μg/lane) was loaded in each lane. The rNIS protein was detectable only in the lactating mammary gland (L-10d). C, Immunohistochemical staining for rNIS expression on mammary glands of lactating rats (L-16 days). Note the intense immunostaining of rNIS on the basolateral membrane of alveolar epithelial cells of lactating mammary glands. Magnification, ×140.

teins were denatured in the denaturing buffer (0.5% SDS and 1% β-mercaptoethanol) for 30 min at 37 C. Denatured proteins were treated with 1 μL (500 U) peptide:*N*-glycosidase F (PNGase F; New England Biolabs, Inc., Beverly, MA) in 50 mmol/L sodium phosphate buffer (pH 7.5) containing 1% Nonidet P-40 at 37 C for 1 h.

Immunohistochemical staining of mammary glands

Immunohistochemical staining was performed as reported previously (17) with some modifications to detect rNIS proteins in the paraffin-embedded mammary gland sections (5 μm). Briefly, the tissue sections were incubated with rNIS polyclonal antibody pAb 716 at a 1:3000 dilution at room temperature for 1 h and then incubated with a 1:200 dilution of biotinylated goat antirabbit IgG (Vector Laboratories, Inc., Burlingame, CA) for 20 min.

Three-dimensional (3-D) histocultures

Specialized collagen gel manufactured from pigskin was purchased from Health Design Industries (Rochester, NY). The dehydrated collagen gel was presoaked in DMEM (Life Technologies, Inc., Gaithersburg, MD) with penicillin (10 units/mL) and streptomycin (10 μg/mL) at 37 C for 2 h and then divided into 12 pieces. At least 1 day before the start of the experiments, each piece was placed in 1 well of a 6-well tissue culture plate containing DMEM with 10% FBS (Life Technologies, Inc.), 0.1 mmol/L nonessential amino acids, and the antibiotics penicillin (10 U/mL) and streptomycin (10 μg/mL). Immediately after surgery or biopsy, human breast tumors in RPMI 1640 medium with penicillin/streptomycin were brought from the Ohio State University hospital tissue procurement to the laboratory. Fat and necrotic tissues were removed, and the remaining tissue was minced with double blades into approximately 1-mm^3 pieces. About 10 of these tumor pieces were placed on each collagen surface. Medium (4 mL) with freshly added hormones was added and changed daily.

Ribonucleic acid (RNA) preparation and RT

Total RNA was extracted from frozen 3-D histocultured breast tumors using TRIzol reagent (Life Technologies, Inc.). The extracted RNA (1 μg) was reverse transcribed using random hexamer primers and Moloney murine leukemia virus reverse transcriptase (Life Technologies, Inc.) in a total volume of 20 μL, and the complementary DNA (cDNA; 1 μL) was used for real-time PCR amplification.

Real-time quantitative PCR

Real-time PCR is a novel technique that measures PCR product accumulation during the exponential phases of PCR reaction. As it allows you to monitor PCR product accumulation cycle by cycle, it provides accurate measurement of target concentration over a wide range of starting materials. Fluorescence-based real-time PCR was carried out on a LightCycler (Roche, Mannheim, Germany) using two independent single-labeled hybridization probes that hybridize adjacently on the PCR product internal to the flanking PCR primers (19). This method is based on the concept that a fluorescence signal is generated if fluorescence response energy transfer (FRET) occurs between two adjacent fluorophores (20). Human (h) NIS-F2 and hNIS-R8 primers (see Fig. 5) were used to amplify the region between nucleotides 681 and 1058 of the hNIS cDNA. The fluorescent probes were designed as shown in Fig. 5. The 3′-labeled 19-mer fluorescein probe (P1) was designed to anneal nucleotides 818–836 of the antisense strand of hNIS cDNA; the fluorescein acts as a donor in FRET and also blocks extension from the probe by *Taq* polymerase. The acceptor (or detection) probe (P2) was 20-mer oligonucleotide, labeled at the 5′-end with LightCycler-Red640 and modified at the 3′-end by phosphorylation to block extension. This acceptor probe binds to the antisense strand of the hNIS at nucleotides 840–859 with a distance of 4 bases 3′ to the donor probe.

All PCR mixtures contained; PCR buffer [final concentration: 10 mmol/L Tris (pH 8.3), 0.25 mg/mL BSA, and 2 mmol/L $MgCl_2$; Idaho Technology, Salt Lake City, UT], 0.25 mmol/L deoxy-NTP (Roche), 0.5 μmol/L of each PCR primer (Molecular Genetics, Huntsville, AL), 0.5 μmol/L of each hybridization probe (Integrated DNA Technologies, Inc., Coralville, IA); and 0.5 U AmpliTaq-DNA polymerase (Perkin-Elmer Corp., Foster City, CA) with 1 μL cDNA/10-μL final volume of reaction mix. The samples were loaded into glass capillary cuvettes (Roche) and centrifuged to place the sample at the capillary after capping. After an initial denaturation step at 95 C for 30 s, conditions for cycling were 40 cycles of denaturation (95 C for 0 s), annealing (56 C for

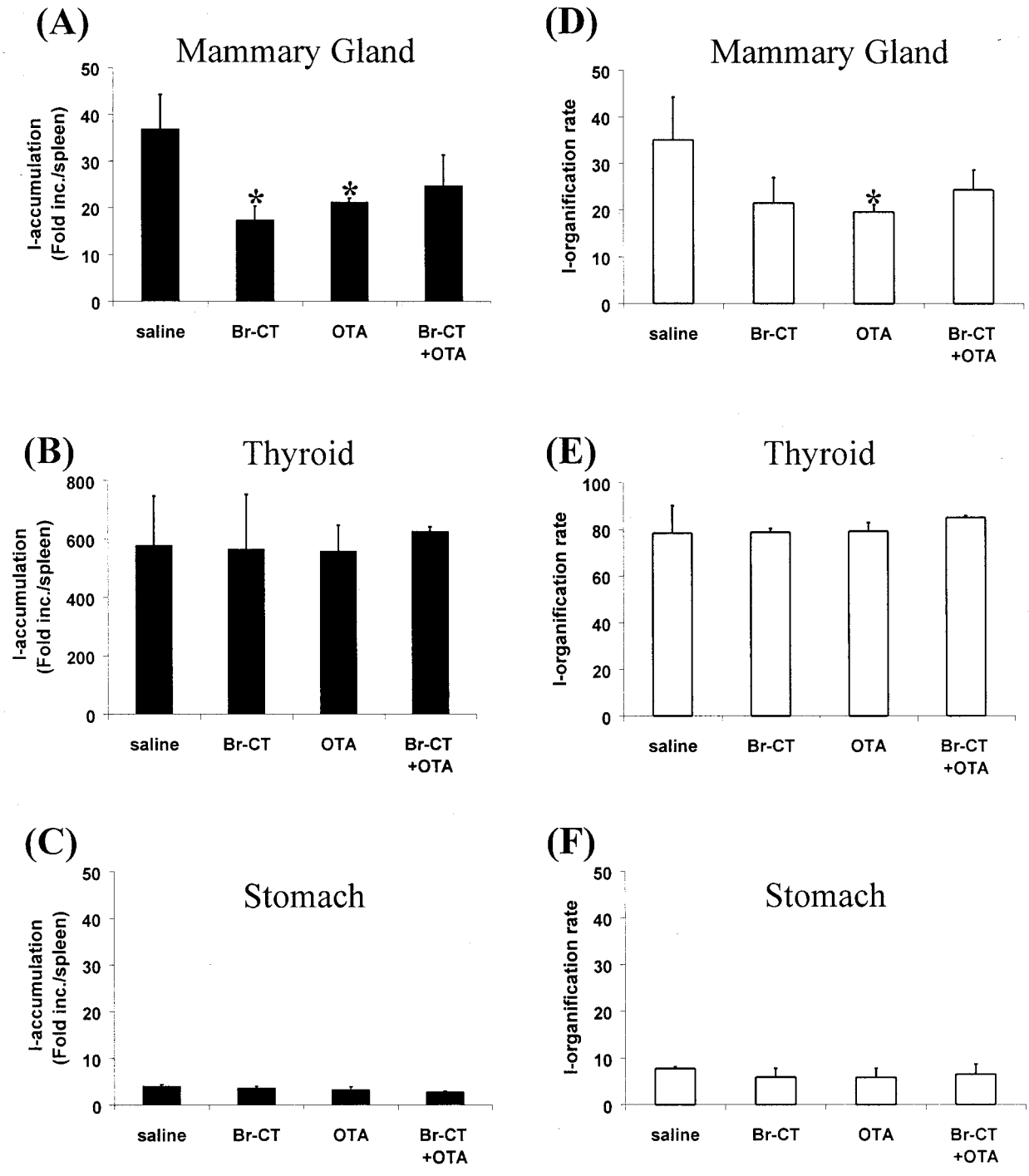


FIG. 3. A–C, $^{125}I^-$ γ-count of the mammary glands (A), thyroid (B), and stomach (C) in lactating rats treated with saline, Br-CT, OTA, or Br-CT plus OTA. Although RAIU in thyroid and stomach were no different among different experimental groups, RAIU in lactating mammary glands decreased 53 ± 8.1% ($P < 0.05$) in Br-CT-treated animals, decreased 42 ± 2.3% ($P < 0.05$) in OTA-treated animals, and decreased 33 ± 17.8% ($P = 0.15$) in Br-CT- plus OTA-treated animals compared to that in saline-treated animals. D–F, Iodide organification rates of the mammary glands (D), thyroid (E), and stomach (F) in lactating rats treated with saline, Br-CT, OTA, or Br-CT plus OTA. Iodide organification rates in thyroid and stomach were not changed by treatments. However, iodide organification rates in lactating mammary gland were decreased in Br-CT-, OTA-, and Br-CT- plus OTA-treated rats. Each experimental group had three rats.

5 s), and extension (72 C for 20 s). The temperature transition rate was programmed at 20 C/s for every transition. Fluorescence was measured at the end of the annealing period of each cycle to monitor amplification. For each run, serial dilutions of hNIS plasmids were used as standards for quantitative measurement of the amount of hNIS cDNA. The data were presented as hNIS copies per mL PCR mixture with 0.1 vol cDNA template. Amplified PCR products were also confirmed by ethidium-bromide agarose gel electrophoresis for its correct size of PCR product. Meanwhile, β_2-microglobulin was amplified to ensure the integrity of the cDNA prepared.

Statistical analysis

Each value represents the mean ± SEM. Statistical evaluation of the results was performed with Student's *t* test, and $P < 0.05$ was considered as significant.

Results

$^{99m}TcO_4^-$ uptake activity is maximal in lactating mammary glands

As $^{99m}TcO_4^-$ is also known to be transported by NIS, $^{99m}TcO_4^-$ whole body scintigraphy was used to evaluate NIS activities in mammary glands at different stages (Fig. 1A). In virgin rats, $^{99m}TcO_4^-$ uptake activity was not detectable in the mammary gland, whereas it was observed in the stomach, thyroid, and bladder (data not shown). In late pregnancy (P-18d), $^{99m}TcO_4^-$ uptake activity in the mammary gland becomes visible by $^{99m}TcO_4^-$ whole body scintigraphy (Fig. 1A). In the lactating mammary gland at 1 day postdelivery (L-1d), $^{99m}TcO_4^-$ uptake activity was evident and was further increased in the lactating mammary gland at 4 days postdelivery (L-4d). However, $^{99m}TcO_4^-$ uptake activity was decreased substantially in involuting mammary gland when the pups were removed from dams for 42 h (I-42h).

A more sensitive and quantitative RAIU assay, $^{125}I^-$ γ-count in isolated tissues, revealed that RAIU in the mammary gland of virgin rat was 2.3 ± 0.9-fold increased over that in spleen (as a control). In the lactating mammary gland at 16 days postdelivery, RAIU was 35 ± 0.4-fold increased over that in spleen (Fig. 1B). In the involuting mammary gland, RAIU was only 5.47 ± 0.53-fold increased over that in spleen (Fig. 1B).

NIS expression is increased in lactating mammary gland

Western blot analysis indicates that two bands with molecular masses of approximately 65 and 75 kDa were detected by an anti-rNIS antibody in lactating rat mammary gland (Fig. 2A). The sizes of rNIS protein in mammary gland were distinct from that of the rNIS protein in thyroid (~90 kDa). However, when the cell lysates of mammary gland and thyroid were treated with PNGase F before Western blot analysis, the nonglycosylated rNIS proteins detected in both mammary gland and thyroid had a molecular mass of about 60 kDa. This suggests that rNIS glycoproteins in mammary gland have a lesser degree of glycosylation than the rNIS glycoproteins in thyroid. Furthermore, it appears that the expression level of rNIS in lactating mammary gland was lower than that in thyroid glands when an equal amount of total protein (100 μg/lane) was loaded on SDS-PAGE (Fig. 2A). In agreement with RAIU found in rat mammary glands of various stages (Fig. 1), rNIS protein is most abundant in lactating mammary gland compared to mammary glands of virgin animals, mammary glands during late pregnancy, and involuting mammary gland (Fig. 2B). The finding that RAIU and rNIS expression were significantly decreased in the involuting mammary gland indicates a quick turnover of rNIS during the involution process.

Cellular localization of rNIS protein in lactating mammary gland was examined by immunohistochemical staining using antibody raised against rNIS. Immunostaining was clearly detected in the basolateral membrane of the alveolar cells (Fig. 2C). When the primary anti-rNIS antibody was substituted with a rabbit IgG, no immunostaining was observed in the mammary glands investigated (data not shown).

RAIU in lactating mammary gland is decreased by treatment with an OTA or Br-CT

To investigate whether OT or PRL hormone has any regulatory effect on RAIU in lactating mammary gland, lactating rats were treated with either a selective OTA, des Gly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT (15), or Br-CT, an inhibitor of PRL release. RAIU in lactating mammary gland was decreased 53 ± 8.1% ($P < 0.05$) in the Br-CT-treated rats and was decreased 42 ± 2.3% ($P < 0.05$) in OTA-treated rats compared to that in saline-injected rats (Fig. 3A). In contrast, RAIU in thyroid (Fig. 3B) and stomach (Fig. 3C) were not affected by Br-CT and OTA treatment, indicating that the effects of Br-CT and OTA were mammary gland specific. However, when the animals were treated with both Br-CT and OTA, no additive effect was observed on RAIU reduction (33 ± 17.8%; $P = 0.15$) compared to that in Br-CT- or OTA-treated rats. Although iodide organification rates in thyroid (Fig. 3E) and stomach (Fig. 3F) were not significantly different among different experimental groups, iodide organification rates in lactating mammary glands were decreased in Br-CT-treated animals (38.8 ± 15.5%; $P = 0.072$) and OTA-treated animals (44.0 ± 4.43%; $P = 0.038$), but not significantly in Br-CT- plus OTA-treated animals (30.4 ± 12.0%; $P = 0.104$; Fig. 3D), compared to those in saline-injected rats. It is noteworthy that the pups were still suckling in all animals studied, and that serum PRL levels were greatly reduced in animals treated with Br-CT or Br-CT plus OTA (see Fig. 4).

OT and hPRL increase the hNIS messenger RNA (mRNA) levels in 3-D histocultures of human breast cancer

To investigate whether OT or hPRL has any direct effect on hNIS expression in human breast tumors, we examined the mRNA level of hNIS in 3-D histocultures of tumor tissues treated with OT or hPRL by real-time quantitative PCR analysis. Forty cycles of amplification were performed with cDNA of each sample, a negative control (no template) and

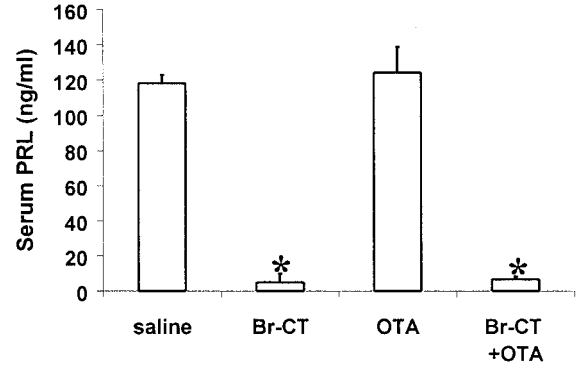
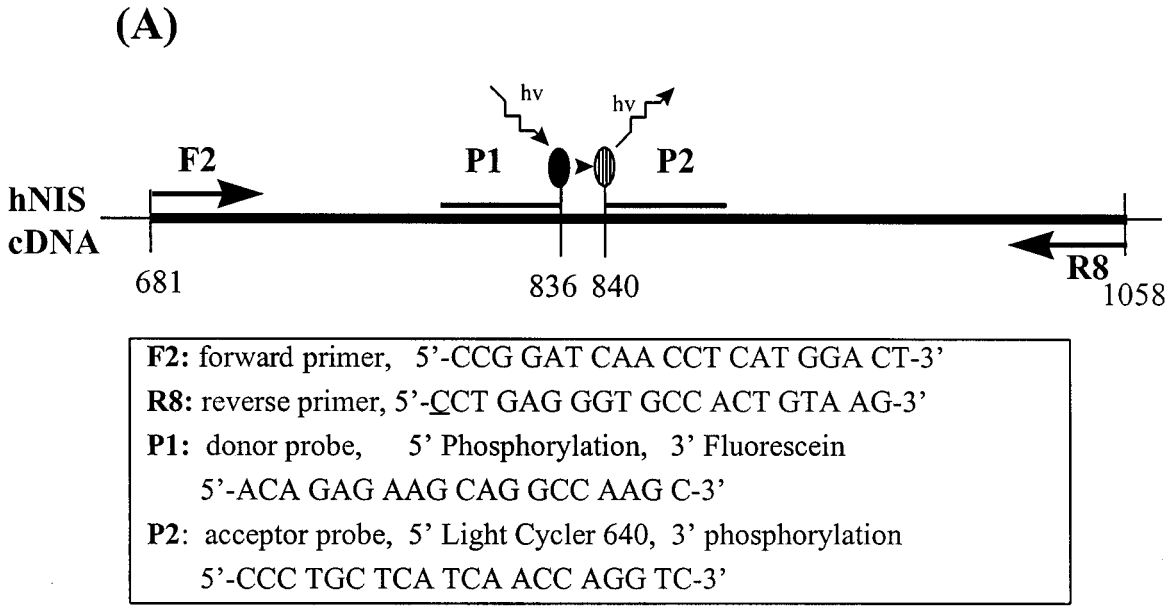


FIG. 4. Serum PRL levels in lactating rats treated with saline, Br-CT, OTA, or Br-CT plus OTA. Serum PRL levels were significantly decreased by 1 week of treatment with Br-CT or Br-CT plus OTA ($P < 0.001$). Each experimental group had three rats.

serially diluted hNIS/PcDNA3 plasmid DNA standards, using the FRET detection system (Fig. 5A). Figure 5B shows fluorescence data for the diluted hNIS/PcDNA3 plasmid DNA standards generated by the LightCycler instrument. No increase in fluorescence signal was observed in the absence of template (Fig. 5B, curve E). The mRNA levels of hNIS were increased in a dose-dependent manner by OT in three of four samples investigated, and by hPRL in two of two samples studied (Fig. 6, A and B). However, the combination of OT and hPRL did not have an additive effect on hNIS expression in the 3-D histocultured breast tumor (Fig. 6B). Instead, the increases in hNIS mRNA were lower than those in OT- or hPRL-treated samples.

Discussion

In this study we showed that NIS expression in mammary gland is modulated by PRL and OT. The NIS expression level is generally correlated with RAIU in rat mammary gland, both of which are maximal during lactation. In lactating mammary gland, rNIS is located at the basolateral membrane of alveolar epithelial cells and is in a less glycosylated form



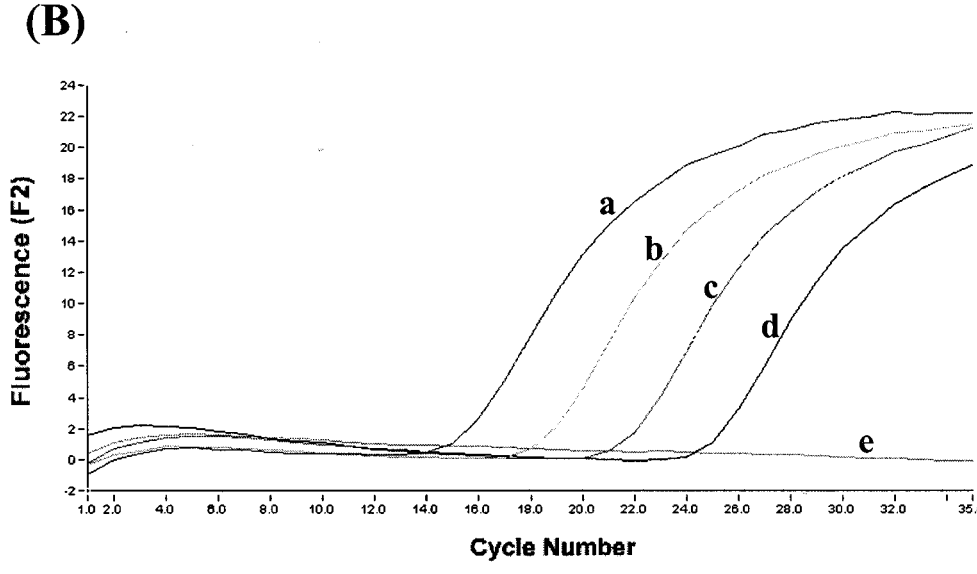


FIG. 5. A, Diagram showing the design of the primers (F2 and R8) and the hybridization probes (P1 and P2) for real-time PCR to quantitate hNIS cDNA. The *bottom box* shows the oligonucleotide sequences of the primers and the hybridization probes. The modified probes are nonextendible oligonucleotides that produce a fluorescence signal by FRET after adjacent hybridization to PCR-amplified fragments. B, Fluorescence signal (F2) *vs.* cycle number for serially diluted hNIS/PcDNA3 plasmid standards and template-free control generated by a LightCycler instrument. a, 6.3×10^9 copies/mL; b, 6.3×10^8 copies/mL; c, 6.3×10^7 copies/mL; d, 6.3×10^6 copies/mL; e, no template.

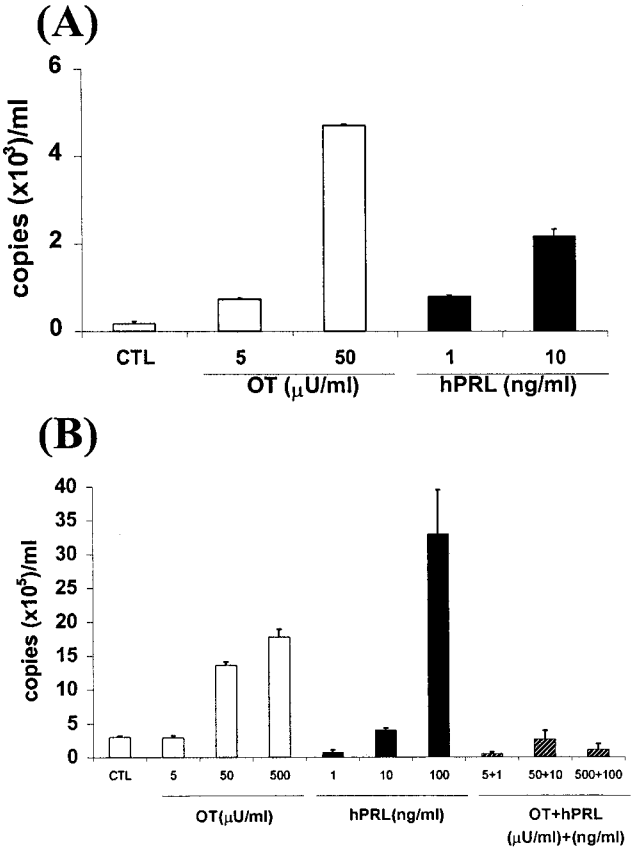


FIG. 6. Real-time quantitative PCR analysis for hNIS mRNA expression in 3D histocultured human breast invasive adenocarcinoma (A) and human breast invasive ductal carcinoma (B) treated with OT, hPRL, or OT plus hPRL. Note that hNIS mRNA levels were increased by OT or hPRL. The combination of OT and hPRL seems to have an antagonistic effect on the increase in hNIS mRNA levels (B). The results shown are the mean ± SEM of two measurements using the LightCycler instrument.

than rNIS in thyroid. RAIU in lactating mammary glands is partially suppressed by OTA or Br-CT, suggesting that NIS expression in breast is modulated by PRL and OT. Indeed, hNIS mRNA levels were increased by OT and PRL in a dose-dependant manner in 3-D histocultured human breast tumor samples.

RAIU activity analyzed by either $^{99m}TcO_4^-$ whole body scintigraphy or $^{125}I^-$ γ-count was maximal in the mammary glands of lactating rats, followed by mammary glands at 42 h involution, and then by mammary glands from pregnant/virgin rats. This result is in agreement with the *in vitro* $^{131}I^-$ γ-count study reported by Maqsood and Peineke (21). In our study we further showed that NIS expression is most abundant in lactating mammary gland. Interestingly, although RAIU activity can be detected *in vivo* in the mammary gland of pregnant rat and involuting mammary gland, Western blot analysis was not sensitive enough to detect NIS expression in the mammary gland of pregnant rat or involuting mammary gland.

We showed that rNIS was mainly detected in the basolateral membrane of alveolar cells and small ductal epithelial cells of lactating rat mammary gland. This finding suggests that iodide is transported from blood to alveolar cells and small ductal cells and then moves to the lumen, where the milk is accumulated and secreted in the mammary gland. In contrast, hNIS was detected in ductal cells, but not in acinar cells, of the human salivary gland (17), suggesting that iodide is added to the saliva in the ductal cells of salivary gland.

The release of PRL was effectively inhibited by Br-CT treatment. Therefore, the modest inhibitory effect of Br-CT on RAIU in lactating mammary gland is not due to suboptimal inhibition of PRL release. However, it is not known whether the effects of oxytocin on NIS expression are fully blocked by OTA treatment, even though a previous study that used similar OTA dosage demonstrated an inhibitory effect on uterine contraction (15). It is possible that the modest inhibitory effect of OTA on RAIU in lactating mammary gland is due to suboptimal inhibition of OT. Furthermore, the inhibitory effects of OTA and Br-CT on RAIU could be masked by a possible long half-life of NIS protein in lactating mammary glands. Finally, it is possible that NIS expression in mammary glands is also modulated by other factors yet to be identified. In any event, our study showed that RAIU in lactating mammary gland is modulated at least in part by OT and PRL.

It has been reported that PRL stimulates iodide accumulation in cultured mammary gland from the midpregnant mouse (22). In this study we showed that PRL increases hNIS mRNA level in a dose-dependent manner in two human breast tumor investigated. It has been shown that the expression levels of genes containing GAS (γ-interferon activation sequence, TTCNNNGAA) are increased by PRL-PRLR signal transduction pathways through the activation of Jak2/Stat5 cascades in breast cells (23). Interestingly, a GAS was identified in the promoter region of hNIS −2880 to −2871 upstream from ATG translational start site (GenBank/EMBL no. AJ132551 and AJ224353).

We have shown that OT increases hNIS mRNA levels in three of four human breast tumors investigated. Interestingly, OTR has been reported to be expressed in 50–90% of the breast cancers derived from glandular or ductal epithelium (12–14). OTR is a G protein-coupled receptor that can activate the G_s-cAMP-protein kinase A (PKA) pathway and/or the $G_{q/11}$-(inositol-1, 4, 5-triphosphate-Ca^{2+})/(diacylglycerol)-protein kinase C (PKC) pathway, depending on ligands and cell types (24). As the stimulation of NIS expression by TSH in thyroid cells is mainly mediated by the G_s-cAMP-PKA pathway, OT may also stimulate hNIS expression in breast tumors by activating the G_s-cAMP-PKA pathway.

Although OT or PRL alone did increase hNIS mRNA levels in some human breast tumors, the combination of OT and PRL seems to have an antagonistic effect on the increase in hNIS mRNA levels. These data indicate that the signaling pathways activated by OT/OTR and PRL/PRLR that stimulate NIS expression in mammary gland may interfere with each other. Indeed, it has been reported that Jak/Stat signaling pathways are inhibited by PKA (25, 26) and PKC (27, 28). It is also possible that OT and/or PRL may stimulate other factors in the breast that, in turn, affect NIS expression. A thorough understanding of the hormonal regulation of NIS expression in breast tissue is required before an optimal

regimen of hormonal stimulation of hNIS expression can be developed to explore the possibility of radioiodide therapy in patients with breast cancer.

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