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Intracellular iodinated compounds affect sodium iodide symporter expression through TSH-mediated signaling pathways

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Abstract. The mechanisms by which thyroid stimulating hormone (TSH) regulates the expression and activity of sodium iodide symporter (NIS) through cAMP-PKA have been partially elucidated by many studies. However, the effects of the TSH-mediated PLC-IP3 signaling pathway on the expression of NIS and how intracellular iodinated compounds interfere with these signaling pathways are poorly understood. In this study, we investigated the effects of the TSH-mediated cAMP-PKA and PLC-IP3 pathways on the expression of NIS in the presence of various intracellular iodinated compounds. The intracellular iodinated compounds were formed by treating cells with different concentrations of iodine with or without methimazole (MMI), an inhibitor of iodine organification, in a pig monolayer thyrocyte *in vitro*. A high concentration of iodine increased NIS expression at the mRNA and protein levels; however, this phenomenon was not observed in the presence of MMI. Both the cAMP-PKA and PLC-IP3 signaling pathways inhibited the expression of NIS at low iodine concentrations; however, in thyrocytes treated with high concentrations of iodine, the effect of cAMP-PKA on the expression of NIS changed from inhibition to promotion, while the PLC-IP3 pathway continued to inhibit NIS expression. These findings indicate that intracellular iodinated compounds affect NIS expression through the TSH-mediated cAMP-PKA and PLC-IP3 pathways.

Introduction

Sodium iodide symporter (NIS), a membrane glycoprotein localized at the basolateral plasma membrane of thyrocytes (1), plays an essential role in the accumulation of iodine in the thyroid. Iodine accumulation is a key step in thyroid hormone synthesis and is crucial in the diagnosis and treatment of thyroid disorders (2). The expression and activity of NIS are mainly regulated by thyroid stimulating hormone (TSH), iodine (3,4) and thyroglobulin (Tg).

TSH, a major regulator of NIS expression and iodine uptake (5), also induces the activity of NIS protein (6,7) through post-translational modifications. TSH increases NIS expression and iodine uptake by positively regulating NIS expression at the protein and mRNA levels through the cAMP-PKA-dependent and -independent pathways (8-10). The withdrawal of TSH results in a decrease in intracellular cAMP concentration and iodine uptake activity (11). TSH not only regulates NIS transcription and biosynthesis, but also mediates its activity through a post-transcriptional mechanism essential for NIS trafficking to the membrane *in vivo* and *in vitro* (6).

Iodine is another important factor regulating thyroid function. In 1948, Wolff and Chaikoff found that high doses of iodine blocked iodine organification in rat thyroid *in vivo*. This phenomenon was later called the Wolff-Chaikoff effect. The function regulated by a high dose of iodine is transient; the thyroid adapts to a sustained high dose of iodine, avoiding the effects of iodine overload and resuming organification (12). The Wolff-Chaikoff effect constitutes a highly specialized intrinsic autoregulatory system that protects the thyroid from high doses of iodine and ensures adequate iodine uptake for hormone biosynthesis (13). The mechanisms underlying the Wolff-Chaikoff effect are complex, and involve a reduction in the expression of NIS at the mRNA and protein levels (12,14).

Methimazole (MMI) is an inhibitor of TPO activity; it inhibits iodine organification and abolishes the iodide uptake-suppressing effect under high doses of iodine (15). This phenomenon shows that the Wolff-Chaikoff effect may be mediated by intracellular or follicular iodinated compounds, such as iodine lipids, which have been confirmed to inhibit TSH-mediated adenylate cyclase activity (16,17).

TSHr is a G protein-coupled receptor that transfers extracellular signals mainly by the TSH/TSHr-cAMP-PKA and TSH/TSHr-PLC-IP3 pathways (18,19). The mechanism by which TSH regulates the expression and activity of NIS through the cAMP-PKA pathway has been partially clarified by several studies. However, the effect of the TSH-mediated PLC-IP3 signaling pathway on the expression of NIS and and how intracellular iodinated compounds interfere with these signaling pathways are not fully understood.

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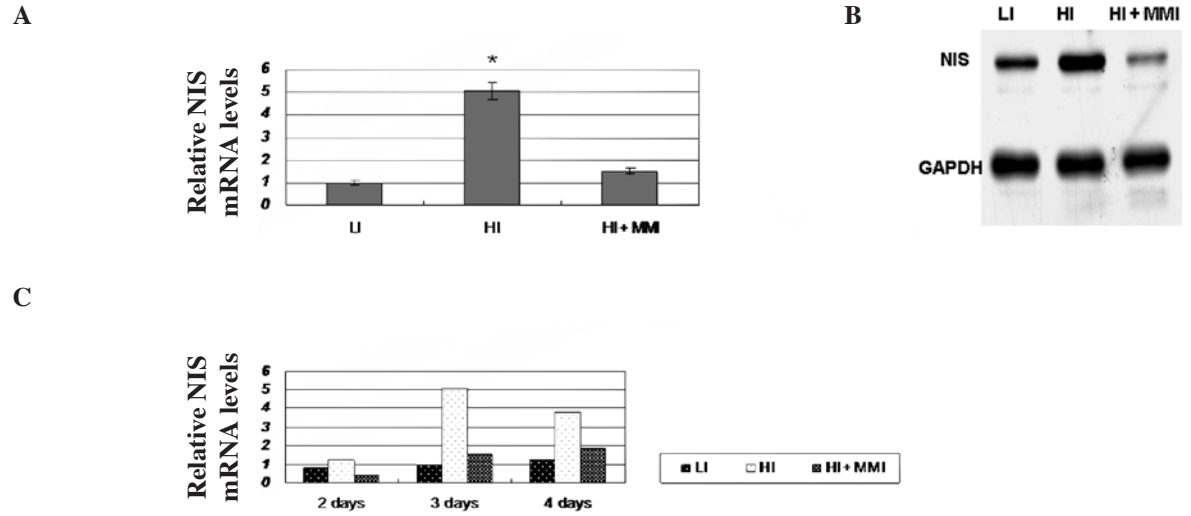

Figure 1. Effects of intracellular iodinated compound on NIS. Pig thyroid primary cells were seeded at a density of $0.2x10^6$ cells/ml and cultured for 3 days, with TSH added after the cells were seeded for 24 h, to form monolayer cells. The monolayer cells were treated with 0.1 μmol/l NaI (LI group), 25 μmol/l NaI (HI group) or 25 μmol/l NaI + 2 mmol/l MMI (HI + MMI group) for 72 h, respectively. Real-time quantitative RT-PCR and Western blotting were performed to measure changes in (A) NIS mRNA and (B) NIS protein levels (normalized to GAPDH). (C) Time-dependent changes in NIS mRNA (n=3). Results are expressed as the mean ± SEM. *P<0.05 compared to LI and HI + MMI.

In this study, we investigated the effect of intracellular iodinated compounds on the regulation of NIS expression and the effects of TSH on NIS expression through the cAMP-PKA and PLC-IP3 pathways in the presence of various intracellular iodinated compounds at different concentrations of iodine, with or without MMI, in a pig monolayer thyrocyte *in vitro*. The aim was to elucidate the effects of different TSH-mediated signaling pathways and intracellular iodinated compounds on the expression of NIS.

Materials and methods

Thyroid monolayer cell culture. The thyroid was removed from a pig within 2 h of its being sacrificed, washed several times with sterilized PBS, and then cut into pieces at a volume of 1 mm^3 under aseptic conditions. Thyroid fragments were digested with 0.125% trypsin (Sigma) for 30 min at room temperature and dispersed into a cell suspension. The suspension was centrifuged at 1,000 rpm for 5 min after being filtered with a 200 mesh filter. The cells were seeded at a density of $0.2x10^6$ cells/ml in DMEM medium (Hyclone) containing 10% bovine serum (Hyclone), 200 IU/ml penicillin (Sigma) and 200 IU/ml streptomycin (Sigma), and maintained at 37˚C with 5% CO_2. TSH (1 mIU/ml) (Merck) was added to the culture medium after the cells were seeded for 24 h.

Administration of iodine and MMI. After TSH treatment and three days of culture, monolayer cells were formed. To investigate the effect of the intracellular iodinated compounds, the monolayers were divided into the high iodine (HI), low iodine (LI) group and high iodine + MMI (HM) groups, treated with 25 μmol/l sodium iodide (Sigma), 0.1 μmol/l sodium iodine and 25 μmol/l sodium iodide (Sigma), and 2 mmol/l MMI (Sigma), respectively.

Administration of H89 and LY294002. H89 and LY294002, blockers of PKA and IP3 activity, are commonly used to study the effects of signaling pathways. To investigate the effects of the TSH-mediated cAMP-PKA and PLC-IP3 pathways, the HI and LI groups were treated with H89 and LY294002 at a final concentration of 30 μmol/l.

Western blot analysis. The cell lysates were centrifuged at 12,000 g for 30 min. Protein concentration was determined by the BCA (Sigma) assay. Cell lysates were then electrophoretically separated on 12% polyacrylamide gels and transferred onto PVDF membranes (Millipore). The membranes were blocked for 1.5 h at room temperature in 5% non-fat milk and incubated overnight at 4˚C with NIS antibodies (Beijing Biosynthesis Biotechnology Co., Ltd.). The membranes were washed three times in TBST for 30 min, incubated with horseradish peroxidase-conjugated secondary antibodies for 45 min, and washed again three times in TBST. Immunoreactive bands were revealed using an enhanced chemiluminescence detection system. The negative group used 2% BSA instead of the primary antibodies. GAPDH (Beijing Biosynthesis Biotechnology Co., Ltd.) was also detected as an internal control. The X-ray film was scanned and the band density was calculated using ImageJ software (20).

Preparation of total RNA and fluorescent quantitative real-time RT-PCR. Total RNA was isolated from the cells using the RNAiso Plus kit (TaKaRa). RNA precipitate was then dissolved in 10-15 μl of RNase-free water and analyzed quantitatively and qualitatively using a spectrophotometer. The integrity of total RNA was determined by 1% formaldehyde agarose gel electrophoresis. A two-step reverse transcription-polymerase chain reaction (RT-PCR) procedure was performed using the PrimeScript™ RT reagent kit (TaKaRa) following the manufacturer's instructions. The resulting cDNA was used in the real-time PCR. For PCR amplification, 2 μl of the cDNA was used in a 20-μl reaction mixture. The PCR primers of NIS were 5'-AGTGATGCTGACGGTTTCTGGGTT-3' and 5'-AGGTTGATCCGGAGTGGTTCTT-3'. Hot-start real-time

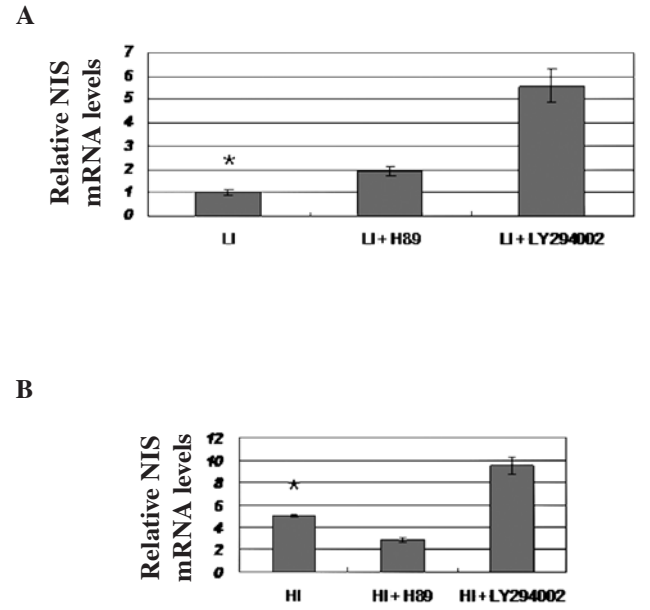

Figure 2. TSH-mediated cAMP-PKA and PLC-IP3 pathways regulate the expression of NIS. Pig thyroid primary cells were seeded at a density of 0.2x10^6 cells/ml and cultured for 3 days, with TSH added after the cells were seeded for 24 h, to form monolayer cells. The monolayer cells were then treated with a PKA blocker (H89) or an IP3 blocker (LY294002) for 72 h under low or high iodine conditions. Real-time quantitative RT-PCR was performed to measure changes in NIS mRNA (normalized to GAPDH). (A) Changes in NIS mRNA in cells treated with 0.1 μmol/l NaI and 30 μmol/l H89 (LI + H89 group), or with 0.1 μmol/l NaI and 30 μmol/l LY294002 (LI + LY group) (n=3). Results are expressed as the mean ± SEM. *P<0.05 compared to the LI + H89 and LI + LY294002 groups. (B) Changes in NIS mRNA in cells treated with 25 μmol/l NaI and 30 μmol/l H89 (HI + H89 group), or with 25 μmol/l NaI and 30 μmol/l LY294002 (HI + LY294002 group) (n=3). Results are expressed as the mean ± SEM. *P<0.05 compared to the HI + H89 and HI + LY294002 groups.

PCR was performed using SYBR green-based detection in a Rotor-Gene 6000, with initiation at 95˚C for 30 sec, then 40 cycles of a two-step PCR comprising 5 sec of denaturation at 95˚C and 30 sec of annealing/elongation at 60˚C. GAPDH was amplified as an internal control using the PCR primers 5'-GAAGGTCGGAGTGAACGGAT-3' and 5'-CATGGGTA-GAATCATACTGGAACA-3'. Data were analyzed using the appropriate expression software.

Statistical analysis. Statistical differences were determined using the Student's t-test. A p-value of <0.05 was considered significant.

Results

Intracellular iodinated compound regulates the expression of NIS. The expression of NIS significantly increased in the HI group compared to the LI group; the effect was abolished when MMI was administered in the HI group (Fig. 1). These results indicate that intracellular iodinated compounds are responsible for the increase in NIS.

TSH-mediated cAMP-PKA and PLC-IP3 signaling pathways regulate the expression of NIS in the presence of various iodine concentrations. When thyrocytes were treated with H89 and LY294002 separately with low concentrations of iodine, the expression of NIS was significantly increased in both the H89 and LY294002 groups; however, this increase was higher in the LY294002 group than in the H89 group (Fig. 2A). With high iodine concentrations, the expression of NIS was significantly decreased in the H89 group, whereas the expression of NIS was still increased in the LY294002 group (Fig. 2B). These results indicate that the TSH-mediated cAMP-PKA and PLC-IP3 pathways have different effects on NIS expression, due to the different intracellular iodinated compounds that are formed by treating cells with different iodine concentrations.

Discussion

Much of the data regarding the effect of iodine on NIS expression and activity is the result of research on the mechanisms of the Wolff-Chaikoff effect. Evidence that an acute high dose of iodine blocks iodide organification by inhibiting the activity of TPO and reduces the expression of NIS at both the mRNA and protein levels has been presented (16,21). A previous study shows that methimazole, an inhibitor of TPO activity, blocks iodine organification and abolishes an acute Wolff-Chaikoff effect (15). These findings reveal that intracellular or follicular iodinated compounds may be responsible for the regulation of NIS expression and iodine uptake. However, results from studies carried out in tissue or animal models cannot exclude the interference of follicular organic iodinated compounds, such as iodinated Tg, which exists within intact thyroid follicle lumen.

In this study, we investigated the effect of intracellular iodinated compounds on the regulation of NIS expression in a pig monolayer thyrocyte *in vitro,* and found that thyroid follicle lumen failed to form and was not capable of storing organic iodinated compounds. High iodine concentrations increased NIS expression at the mRNA and protein levels. This phenomenon was not observed with the addition of MMI (Fig. 1A and B). These effects on regulation were similar on the 2nd, 3rd and 4th day after the monolayer cells were treated with drugs (Fig. 1C). Our findings indicate that intracellular iodinated compounds are responsible for the increase in NIS expression.

We then investigated the effect of the TSH-mediated cAMP-PKA and PLC-IP3 pathways on the expression of NIS by inhibiting the activity of PAK or IP3 in the presence of different iodine concentrations. Both the cAMP-PKA and PLC-IP3 signaling pathways inhibited the expression of NIS with low concentrations of iodine, though the effect of the PLC-IP3 pathway was more marked than that of the cAMP-PKA pathway. The inhibition of IP3 activity by LY294002 resulted in higher NIS expression than the suppression of PKA activity by H89 (Fig. 2A).

Of note, the effect of the cAMP-PKA pathway on NIS expression changed from inhibition to promotion with high concentrations of iodine. The expression of NIS was significantly decreased rather than increased compared to the control group when APK activity was inhibited by H89 (Fig. 2B).

We showed that intracellular iodinated compounds promote NIS expression by altering the effects of the TSH-mediated cAMP-PKA and PLC-IP3 pathways, and that high intracellular iodinated compounds reversed the effect of the TSH-mediated

cAMP-PKA pathway on the expression of NIS, from inhibition to promotion. This finding may explain the observed up-regulation of NIS when a high dose of iodine was administrated. It also strongly supports the notion that intracellular iodinated compounds disturb TSH-mediated adenylate cyclase activity, as indicated by recent studies (16,22).

The results of the present study contradict the reported results obtained using tissue and animal models possessing thyroid follicles. This discrepancy may be attributed to follicular Tg, which has been confirmed to inhibit thyroid-specific genes and iodine uptake activity (23), and to regulate follicular function through negative feedback (24).

The incidence of thyroid dysfunction and autoimmune thyroid diseases caused by excessive iodine has significantly increased (25,26). The present findings may shed the light on the pathogenesis of these thyroid diseases.

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