EXPERIMENTAL STUDY

Regulation of the sodium iodide symporter by iodide in FRTL-5 cells

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Abstract

Objective: The acute decrease in iodide organification in the thyroid in response to excess iodide is termed the acute Wolff–Chaikoff effect and normal organification resumes in spite of continued high plasma iodide concentrations (escape from the acute Wolff–Chaikoff effect). We have recently reported that large doses of iodide given to rats chronically decrease the sodium/iodide symporter (NIS) mRNA and protein, suggesting that escape is due to a decrease in NIS and subsequent iodide transport. We have now studied the effect of excess iodide on NIS in FRTL-5 cells to further explore the mechanisms whereby excess iodide decreases NIS.

Design: FRTL-5 cells were employed and were incubated in the presence or absence of various concentrations of iodide. NIS mRNA and protein and the turnover of NIS were assessed.

Methods: NIS mRNA was measured by Northern analysis, NIS protein by Western analysis and NIS turnover by pulse–chase labeling experiments.

Results: Iodide (10^{-3} mol/l) had no effect on NIS mRNA in FRTL-5 cells at 24 and 48 h compared with cells cultured in the absence of iodide. However, excess iodide decreased NIS protein by 50% of control values at 24 h and by 70% at 48 h. This effect of iodide was dose dependent. Pulse–chase experiments demonstrated that there was no effect of iodide on new NIS protein synthesis and that the turnover of NIS protein in the presence of iodide was 27% faster than in the absence of added iodide.

Conclusions: Excess iodide does not decrease NIS mRNA in FRTL-5 cells but does decrease NIS protein, suggesting that in this *in vitro* thyroid cell model iodide modulates NIS, at least in part, at a post-transcriptional level. This iodide-induced decrease in NIS protein appears to be due, at least partially, to an increase in NIS protein turnover.

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Introduction

The Wolff–Chaikoff effect refers to the iodide-induced inhibition of iodide organification and subsequent hormone synthesis within the thyroid and was first reported in 1944 (1). The effect has been postulated to be mediated by intermediate organic iodocompounds formed within the thyroid (2) as a result of high intrathyroidal iodide concentrations. After a period of 1–2 days, the thyroid adapts or escapes from this inhibition (3), and this is referred to as the escape from or adaptation to the acute Wolff–Chaikoff effect. We had proposed that the escape mechanism was due to a decrease in iodide transport from the plasma into the thyroid (4). This would lead to a reduction in intrathyroidal iodide concentrations that would be insufficient to sustain the decreased organification of iodide. Recently, we reported that

the escape from the acute Wolff–Chaikoff effect was associated with a decrease in the thyroid sodium/iodide symporter (NIS) mRNA and protein (5). Thyroid NIS mRNA decreased as early as 6 h after administration of iodide to rats and NIS protein decreased as early as 24 h. We postulated that the mechanism of NIS regulation was likely to be, at least in part, transcriptional in this *in vivo* model.

FRTL-5 cells are a thyroid-stimulating hormone (TSH) dependent rat thyroid cell line that has been shown to be a useful system to examine thyroid cell iodide transport *in vitro* (6). It has been shown that TSH stimulation of iodide transport in this cell line is partly due to a rapid increase in NIS gene expression, followed by a relatively slow NIS protein synthesis (7). To further study the mechanism(s) whereby iodide affects NIS *in vitro*, we examined the effects of iodide on NIS mRNA and NIS protein in FRTL-5 cells.

Materials and methods

Cell culture

FRTL-5 cells were obtained from ATCC, and were grown in F12 K media (Gibco BRL Inc., Grand Island, NY, USA) and supplemented with 5% calf serum and 6H, a mixture of the following six hormones: 10 µg/ml insulin (Sigma-Aldrich, Inc., St Louis, MO, USA), 5 µg/ml transferrin (Sigma Inc.), 10 ng/ml glycyl-L-histidyl-L-lysine acetate (Sigma Inc.), 10 ng/ml somatostatin (Sigma Inc.), 0.4 ng/ml cortisol, and 10 mU/ml bovine TSH (Sigma Inc.). The cells were maintained in a 5% CO₂ and 95% air atmosphere at 37 °C with a media change every 3–4 days and passage every 7 days.

RNA analysis

Total RNA was extracted from cells using a modification of the method of Chomczynski and Sacchi (Tri-Reagent; Sigma Inc.). Northern analysis was carried out as previously described (5). Membranes were sequentially hybridized with ³²P-labeled NIS and cyclophilin cDNA probes and analyzed using a phosphorimaging system (Molecular Dynamics, Inc., Sunnyvale, CA, USA). Rat NIS cDNA probe and antibody were kindly provided by Dr Nancy Carrasco, Albert Einstein College of Medicine, New York, NY, USA.

Protein analysis

Total protein from the cells was extracted as follows. Cells were lysed in RIPA buffer (1×PBS, 1% Nonidet P-40, sodium deoxycholate, 0.1% SDS) with protease inhibitors (aprotinin, sodium orthovanadate, PMSF) added. Western analysis was carried out as previously described (5). Briefly, 15 µg of protein per lane were loaded on SDS polyacrylamide gel and subjected to electrophoresis at constant voltage (150 V). Electrophoresis to a nitrocellulose membrane was performed for 2 h using a semi-dry electroblotting system. Blocking was done overnight with TTBS/milk (TBS, 1% Tween, 5% milk). The membrane was incubated with a 1:5000 dilution of affinity purified NIS antibody, washed four times, incubated with a 1:25 000 dilution of a horseradish peroxidase conjugated antibody (Pierce, Inc., Rockford, IL, USA), and washed again. An enhanced chemiluminescent substrate was added and the membrane was exposed to film. Quantification was by densitometry (Molecular Dynamics, Inc.).

Pulse–chase labeling experiments

FRTL-5 cells were depleted of methionine by washing twice followed by incubation for 1 h with methionine-free RPMI 1640 media (Gibco Inc.) supplemented with 6H. Cells were pulsed with 350 µCi/ml of ³⁵S methionine in RPMI 1640 media supplemented with

6H for 30 min. In some experiments iodide was added to the pulse media for the iodide-treated cells at a concentration of 10^{−3} mol/l. This was followed by chase with F12 K media supplemented with 6H and 10X cold methionine for periods varying from 0 to 24 h. Iodide (10^{−3} mol/l) was added to the chase media for the iodide-treated cells in all experiments. Total protein was extracted as described above.

Immunoprecipitation

Total protein (450 µg) in 1 ml of lysate was incubated with 1 µg of anti-NIS antibody for 2 h at 4 °C with rotation. As a control, the lysate was incubated with the preimmune serum. This was followed by incubation with protein A/G agarose (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) for 2 h at 4 °C for 2 h with rotation. After centrifugation at 1000 *g*, the pellet was washed four times with RIPA buffer. After the last wash, the pellet was suspended in 35 µl of lamelli buffer, incubated for 30 min at 37 °C and resolved on a 8% SDS acrylamide gel. The gel was fixed with 12% acetic acid, 50% methanol for 30 min and soaked with a scintillation solution (En³hance; NEN Research Products Inc., Boston, MA, USA) for a further 30 min. After extensive washing, the gel was dried on a gel dryer and exposed to X-ray film at −70 °C for up to 48 h.

Statistical analysis

Statistical analysis was performed using a statistical analysis program (InStat v 2.02; GraphPad Software, Inc., San Diego, CA, USA). The Student's *t*-test was used to analyze the difference between the iodide and control groups of cells. *P* < 0.05 was considered to be significant.

Results

Effect of NaI on NIS mRNA and protein in FRTL-5 cells

Compared with control values, exposure of FRTL-5 cells to NaI (10^{−3} mol/l) did not affect the level of NIS mRNA at 24 or 48 h (Fig. 1a, b). NIS mRNA was higher at 48 h compared with 24 h, due to the stimulatory effect of TSH on NIS mRNA, but excess iodide did not affect this TSH-induced increase. Further incubation of FRTL-5 cells with varying concentrations of NaI had no effect on NIS mRNA (data not shown). By contrast, in FRTL-5 cells exposed to NaI (10^{−3} mol/l), NIS protein decreased to 50% of control levels at 24 h and to 30% of control levels at 48 h (Fig. 2a, b). Increasing concentrations of NaI also progressively decreased the amount of NIS protein (Fig. 3).

Figure 1 Northern blot analysis NIS mRNA in FRTL-5 cells exposed to excess iodide. (a) Autoradiograph of a representative Northern blot of total RNA extracted from FRTL-5 cells incubated with 10^{-3} mol/l iodide and controls for 24 and 48 h. The blot was hybridized with a ^{32}P -labeled NIS cDNA probe and exposed to film. (b) NIS mRNA levels in FRTL-5 cells incubated with 10^{-3} mol/l iodide vs controls as determined by Northern blotting. Band density was measured using a phosphorimager. Results are pooled from three separate experiments, each done in duplicate or triplicate and are normalized with respect to individual FRTL-5 cyclophilin mRNAs. The means $\pm$ S.E.M. of each group are shown. A *P* value of <0.05 is significant.

Treatment of FRTL-5 cells with TSH and NaI

It is known that TSH is the major stimulator of NIS mRNA and protein expression (7, 8). We have found that iodide suppresses NIS protein expression both *in vivo* (5) and *in vitro*. This experiment was performed to determine whether iodide could block the TSH-induced increase in NIS protein expression. FRTL-5 cells were grown in the absence of TSH for 72 h. The medium was then changed and TSH (10 mU/ml) and NaI (10^{-3} mol/l) added. An increase in NIS protein was observed in TSH-treated cells incubated both with and without iodide; however, the increase was less in the iodide-treated cells at 24 and 48 h (Fig. 4).

Pulse–chase labeling experiments

In order to explore further the mechanism of the iodide-induced decrease of NIS protein in the absence of a change in NIS mRNA, pulse–chase labeling experiments were carried out to determine whether iodide alters NIS protein turnover in FRTL-5 cells. Two protocols were used. In the first protocol, iodide-treated cells were both pulsed and chased with iodide in the medium. Two bands were observed at the 1-h chase, the smaller band of approximately 50 kDa representing the unglycosylated NIS precursor protein and the larger

Figure 2 Western blot analysis of NIS protein in FRTL-5 cells exposed to excess iodide. (a) Autoradiograph of a representative Western blot of protein extracted from FRTL-5 cells incubated with iodide (10^{-3} mol/l) and controls for 24 and 48 h. The blot was hybridized with a primary anti-NIS antibody and a secondary horseradish peroxidase conjugated antibody. Protein detection was by enhanced chemiluminescence. (b) NIS protein levels in FRTL-5 cells incubated with iodide (10^{-3} mol/l) vs controls as determined by Western blotting. Band density was measured using a densitometer. Results are pooled from three experiments, each done in duplicate or triplicate and are expressed as densitometry units. The means $\pm$ S.E.M. of each group are shown. A *P* value of <0.05 is significant.

Figure 3 Autoradiograph of a representative Western blot of protein extracted from FRTL-5 cells incubated with 10^{-6} to 10^{-3} mol/l iodide for 24 h.

Figure 4 Western blot analysis of protein from FRTL-5 cells incubated without TSH, and then with TSH and NaI. Control, 0 h (lane 1) was in the absence of TSH. TSH was then added and cells were harvested at 8, 24 and 48 h (lanes 2–6). Iodide (10^{-3} mol/l) was also added to cells (lanes 2, 4 and 6).

Figure 5 Representative autoradiograph of a pulse-chase of NIS protein in FRTL-5 cells in the absence and presence of iodide. All the samples were pulsed with ^{35}S -methionine for 30 min. The control samples in lanes 2, 4, 6 and 8 were chased with media and excess cold methionine. The samples in lanes 3, 5, 7 and 9 were chased with media and excess cold methionine in the presence of iodide (10^{-3} mol/l). The sample in lane 1 was incubated with preimmune serum. The samples in the other lanes were incubated with anti-NIS antibody. Two bands are visible at the 1-h chase representing the unglycosylated (lower band) and glycosylated (upper band) forms of NIS. The lower band disappears and only the upper band persists after 8 h of chase.

Table 1 Half-life of NIS protein in the presence or absence of excess iodide.

	Half-life of cells in the absence of iodide (h)	Half-life of cells incubated with 10^{-3} M iodide (h)	Percentage difference in half-life
Experiment 1	16.7	11.7 (iodide in pulse and chase media)	30%
Experiment 2	20.3	15.5 (iodide in pulse and chase media)	24%
Experiment 3	12.7	9.4 (iodide in chase media only)	26%

band of approximately 86 kDa representing the fully processed glycosylated NIS protein (Fig. 5). Fairly rapid glycosylation was observed, resulting in complete disappearance of the smaller band by the 8-h chase. This is similar to the results examining the biogenesis of NIS described previously (8). The normal half-life of NIS in these experiments was variable but less than 24 h (12.7–20.3 h). In both protocols, the half-life of newly synthesized NIS protein in the iodide-enriched cells was consistently less (approximately 27%) than that of the control cells, suggesting that iodide enhances NIS protein turnover (Fig. 5, Table 1). To determine if iodide also had an effect on new NIS protein synthesis, cells were pulsed with iodide but without performing a chase incubation. The iodide-treated cells had a similar amount of NIS protein compared with controls, suggesting that iodide had no effect on translation (Fig. 6, Table 2).

Figure 6 Autoradiograph of NIS protein from FRTL-5 cells pulsed with ^{35}S -methionine for 30 min. The sample in lane 1 was incubated with preimmune serum and the samples in lanes 2–5 were incubated with anti-NIS antibody. The samples in lanes 2 and 4 were controls. The samples in lanes 3 and 5 were pulsed in the presence of 10^{-3} mol/l iodide.

Discussion

We recently reported that the escape from the acute Wolff–Chaikoff effect in rats was associated with a decrease in NIS mRNA and protein (5). In the dog, iodide administration has also been reported to decrease NIS mRNA, but NIS protein was not measured (9). We chose the FRTL-5 cell line to further evaluate the escape from the Wolff–Chaikoff effect *in vitro*. The FRTL-5 cell line is a non-transformed rat thyroid cell line that has proved extremely useful in the study of thyroid cell biology. In this system, the addition of TSH stimulates iodide transport which is mediated by cAMP and is dependent on protein synthesis (10). An iodide-induced decrease in iodide uptake, consistent with the proposed mechanism of the escape phenomenon, has also been reported in these cells (11). Dai *et al.* cloned the rat NIS cDNA in 1996 and reported that it encodes an intrinsic membrane protein (12). More recently, Kogai *et al.* showed that FRTL-5 cells expressed NIS and that the TSH stimulation of iodide transport in these cells was due to a rapid increase in NIS gene expression followed by relatively slow protein synthesis (7).

We observed that iodide decreased both NIS mRNA and protein *in vivo* and postulated that the mechanism was likely to be, at least in part, transcriptional (5). However, our observations in FRTL-5 cells *in vitro* were different. Contrary to expectations, there was no decrease in NIS mRNA in cells incubated with 10^{-3} mol/l iodide compared with corresponding controls. We varied the concentrations of iodide from 10^{-6} to 10^{-3} mol/l, and the incubation time from 6 to 72 h,

Table 2 Quantitation of newly formed NIS protein in the presence or absence of excess iodide.

	Lower band (relative densitometry units)	Upper band (relative densitometry units)	Combined upper and lower bands (relative densitometry units)
Control	1	1	1
Control	0.8	0.77	0.8
Iodide (10^{-3} M)	1.08	0.93	1.08
Iodide (10^{-3} M)	0.78	1.01	0.8

but no difference in the levels of NIS mRNA compared with controls was observed (data not shown). This finding is similar to those of others (Masato Ikeda, Yamansahi Medical University, personal communication) although it differs from that observed by Spitzweg *et al.* who reported a 50% decrease in NIS mRNA in FRTL-5 cells incubated with 10^{-4} mol/l iodide (13). It is unlikely that this discrepancy is due to different incubation conditions as we did not observe any effect of iodide on NIS mRNA despite varying the concentration of iodide and the time of incubation. It is possible that the different sublines of the FRTL-5 cells used in the present study may account for the observed lack of effect of iodide on NIS mRNA.

However, we did observe a striking decrease of NIS protein in FRTL-5 cells induced by 10^{-3} mol/l iodide. In the cells incubated for 24 h, a 50% decrease was observed while in those cells incubated for 48 h, a 70% decrease was observed. This time-dependent decrease in NIS protein in iodide-treated cells is consistent with the time-dependent decrease in iodide uptake reported by Grollman *et al.* (11). We also observed a linear dose-dependent decrease in NIS protein with increasing concentrations of iodide from 10^{-6} to 10^{-3} mol/l, again very similar to the decrease in iodide uptake with increasing concentrations of iodide (11). Thus, in FRTL-5 cells, NIS protein is decreased by iodide in a time- and dose-dependent manner that is mediated at the post-transcriptional level and possibly related to increased protein turnover.

The half-life of NIS in FRTL-5 cells has been reported to be far greater than 48 h (8) and perhaps as long as 4 days (14). It is unclear why our observed half-life of NIS protein of less than 24 h in FRTL-5 cells differs significantly from that previously reported. Again, it is possible that different sublines of FRTL-5 cells may vary in properties such as degree of iodination and NIS protein half-life and this may account for the observed differences. From our observations both in rats *in vivo* and in FRTL-5 cells *in vitro*, iodide decreased NIS protein within 24 h. Such a rapid effect is unlikely to be observed if the half-life of NIS was greater than 24 h, suggesting that NIS protein turnover may indeed be faster than previously reported. We also observed that iodide decreased the half-life of NIS by approximately 27%. This increase in NIS protein turnover is likely to be partially responsible for the significant iodide-induced decrease in NIS protein seen at 24 h. We did

not find any evidence that iodide had a significant effect on NIS protein translation, although this could be another mechanism by which iodide decreases NIS protein.

TSH is a potent stimulator of NIS mRNA and protein *in vivo* (8) and *in vitro* (7) and stimulates NIS via a novel cAMP sensitive pathway (15). Ohmori *et al.* reported that a novel transcription factor was essential for TSH induced up-regulation of NIS gene expression and that it was distinct from thyroid transcription factor-1, thyroid transcription factor-2 or PAX-8 (16). When 1 mU/ml TSH was added to quiescent cells grown in the absence of TSH for 8 days, NIS mRNA increased significantly at 3–6 h and reached a peak approximately six times basal level at 24 h (7). NIS protein only increased after 36 h and took 72 h to peak. We observed that iodide had a striking effect on decreasing NIS protein both *in vivo* (5) and *in vitro* and that the addition of iodide decreased the TSH stimulated increase in NIS protein expression in quiescent cells. While the increase in NIS protein was markedly less in the iodide-treated cells compared with controls, iodide was unable to completely block the TSH-stimulated increase in NIS protein, suggesting that there was continued protein synthesis even in the presence of excess iodide.

Iodide is likely to affect NIS protein expression via several different mechanisms. There is a direct effect of iodide on decreasing NIS mRNA *in vivo*, while we have observed that iodide decreases NIS protein independent of NIS mRNA levels *in vitro*. This effect of iodide on decreasing NIS protein *in vitro* is both time- and dose-dependent and is possibly explained by an increase in NIS protein turnover. It is possible that these mechanisms are not mutually exclusive and that iodide may have direct effects on NIS mRNA as well as NIS protein *in vivo*. Iodide induces both the acute Wolff–Chaikoff effect as well as its escape by multiple mechanisms that are increasingly becoming understood. Future experiments will hopefully more fully define the exact mechanisms of the escape from the acute Wolff–Chaikoff effect.

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