The sodium iodide symporter gene and its regulation by cytokines found in autoimmunity

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Abstract

Iodide concentration by the thyroid gland, an essential step for thyroid hormone synthesis, is mediated by the Na+/I− symporter (NIS). To identify factors that may regulate this process, we have studied NIS gene expression in the Fisher rat thyroid cell line (FRTL-5) by a semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) technique. Increasing concentrations of bovine TSH (0·1, 1, 10, 50 and 100 mU/l), with or without tumour necrosis factor-α (TNF-α), interferon-γ (IFNγ) or interleukin-1α (IL-1α) were added to FRTL-5 cells previously deprived of TSH for a minimum of 5 days. RNA was extracted and samples were studied for NIS expression. TSH enhanced NIS mRNA expression in a dose-dependent manner, with induction evident at 0·1 mU/l, reaching a peak at 50 mU/l, an effect detected after 6 h of stimulation, but not in the first 2 h. Both TNFα and, to a lesser extent, IL-1α inhibited basal and TSH-induced NIS expression. High concentrations of IFNγ also downregulated TSH-stimulated NIS mRNA expression.

Using the same technique, we also investigated NIS mRNA tissue distribution in two male and one female Wistar rats. High levels of NIS expression were detected in the thyroid, stomach, and mammary gland, lower levels were found in the intestine, adipose tissue and liver, borderline levels were expressed in the salivary gland, and no expression was detected in the kidneys.

In summary, we have shown that TSH upregulates rat NIS gene expression in vitro, and this induction can be modulated by cytokines. Analysis of the distribution of rat NIS mRNA ex vivo demonstrated variable levels of NIS transcription in different tissue samples.

Introduction

Iodide (I−) uptake by the thyroid gland is an essential step for the formation of thyroid hormones and is mediated by the sodium/iodide symporter (NIS). The NIS is localised to the basolateral membrane of thyroid follicular cells (TFC) transporting extracellular I− using the sodium (Na+) gradient generated by the Na+/K+ ATPase as an energy source (Chambard et al. 1983). Although the physiological properties of the NIS are well characterised, its amino acid sequence was described only recently (Carrasco 1993, Dai et al. 1996, Smanik et al. 1996). Studies on NIS gene expression have been limited, and to date little is known concerning the factors that modulate NIS mRNA expression. In addition, iodide transport is not exclusive to the thyroid gland, as it is also found in other organs including the gastric mucosa, mammary and salivary glands (Halmi 1961), and the relative NIS gene expression in these tissues remains to be investigated.

Several lines of evidence suggest that cytokines have an important role in autoimmune hypothyroidism and Graves’ disease. These molecules, produced by both the infiltrating inflammatory cells and the TFC in autoimmune thyroid disease (Bagnasco et al. 1987, Del Prete et al. 1989, Zheng et al. 1992, Watson et al. 1995, Ajjan et al. 1996), can induce immunological changes in TFC in addition to affecting their growth and function (Todd et al. 1985, Weetman & Rees 1988, Tandon et al. 1992, Vargas et al. 1994, Tominaga et al. 1991, Huber & Davies 1990, Rasmussen et al. 1994, Pang et al. 1993). Previous studies have shown that cytokines can modulate iodide uptake of TFC by mechanisms that remain unclear (Pang et al. 1993, Rasmussen et al. 1994). In the present study, we have investigated NIS tissue distribution in Wistar rats in a variety of tissues, and the level of NIS gene expression was evaluated using a semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) technique. We have also studied the effect of IL-1α, IFNγ and TNFα.
on NIS mRNA expression in FRTL-5 cells, a rat thyroid cell line, using the above technique.

**Materials and Methods**

**Tissue samples**

The thyroid, mammary gland, stomach, intestine, liver, salivary gland, kidney, orbital fat, shoulder fat, epididymal fat and visceral fat were excised from two male and one female lactating Wistar rats. Kidney, mammary gland, orbital, shoulder and epididymal fat tissues were analysed from one animal. Thyroid, stomach, intestine, liver and visceral gland tissues were taken from three rats. Tissue samples were immediately snap frozen in liquid nitrogen and kept in liquid nitrogen until required for analysis.

**FRTL-5 cell culture**

FRTL-5 cells, passed up to 20 times, were obtained from Dr L Kohn (NIH, Bethesda, MD, USA) in 1994. These cells were grown in 24-well plates as previously described (Weiss et al. 1984a,b), except that thyroid-stimulating hormone (TSH) was used at concentrations of 1 U/l. When cells reached 70% confluence, they were deprived of TSH for a period of 5–7 days. Cells were then treated with 100 mU/l of either bovine (b) TSH (preparation 53/11, National Institute for Biological Standards and Control, Potters Bar, Hertfordshire) or human (h) recombinant TSH (Sigma, Poole, Dorset, UK) and RNA was extracted after 0·5, 1, 2, 6, 12 and 24 h of stimulation, as described below. RNA was also extracted after the application of increasing concentrations of TSH (0·1, 1, 10, 50 and 100 mU/l) for a period of 12 h. Both TSH-stimulated and TSH-deprived FRTL-5 cells were further treated with IL-1α (10, 100 or 1000 U/ml; kindly provided by Hoffman-La Roche Inc., Nutley, NJ, USA), IFNγ (10, 100 or 1000 U/ml; Boehringer Mannheim Ltd, Lewes, East Sussex, UK) or TNFα (10, 100 or 1000 U/ml; Calbiochem, Nottingham, UK) and RNA was extracted after 12 h of stimulation. Experiments were repeated with two different cultures, and PCR were performed at least twice on each set of cell cultures. Iodide uptake experiments were also performed in cells stimulated with bTSH or hTSH with or without the addition of cytokines.

**RNA extraction and cDNA synthesis**

RNA was extracted from thyroid cell culture and whole tissue (3–50 mm3 pieces) by using TRIZol (Gibco, Paisley, UK) according to the manufacturer’s protocol. Moloney murine leukaemia virus reverse transcriptase (MMLV-RT) was used to synthesize cDNA as described previously (Ajjan et al. 1996).

**PCR amplification**

Duplex PCR was performed in 50 µl amplification reaction which contained: 5 µl 10 × concentrated buffer (10 mM MgCl2, 100 mM Tris–HCl pH 8·3, 0·1% gelatin, 500 mM KCl, 1% Tween 20, 1% Nonidet NP40; all from Sigma), 2·5 U Taq DNA polymerase (Promega, Southampton, UK), 0·1 µM each dNTP, 1·5 µl cDNA, and 40 µl autoclaved, deionised water.

Amplifications were carried out using cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min. In addition to the NIS primers, β-actin primers were used in the same reaction, to enable semi-quantitation, and to provide a control for the integrity of the cDNA. Initial experiments were carried out to optimise the PCR conditions using a variety of primer and magnesium concentrations. We have also applied different PCR cycles, ranging from 26 to 30 and 22 to 24 cycles for NIS and β-actin respectively, to ensure the linearity of the amplification reactions. For tissue culture, 23 cycles were used to amplify β-actin, whereas 26–28 for TSH-stimulated and 28–30 cycles for TSH-deprived cells were used to amplify NIS-specific PCR product. Additional house-keeping genes, 7B6 and glyceraldehyde phosphate dehydrogenase (GAPDH), were also tested using 23–25 cycles for the former and 26–28 cycles for the latter. For tissue samples, 23 cycles for β-actin and 27 cycles for NIS were used except for adipose tissue from the orbit, shoulder and epididymis, for which 29 and 33 cycles were used for β-actin and NIS amplification respectively. Samples negative for NIS expression using 25–28 cycles were further subjected to 35–37 cycles of PCR amplification.

The oligonucleotide primers and probe of NIS were designed according to sequences published earlier (Dai et al. 1996, Ajjan et al. 1996). The sequences were as follows: NIS primer sense, 5’ CTG CGA CTC TCC CAC TGA 3’; NIS primer antisense, 5’ CGC AGC TCT AGG TAC TGG TA 3’; NIS probe, 5’ GGC AAC ACG CCG TAG TCC 3’; β-actin sense, 5’ GTG GGG CCC GCC AGG CACCA 3’; β-actin antisense, 5’ CTC CTG GAG TCC TTC TGG TAG CAG CAC GAT TCC 3’; β-actin probe, 5’ CCG GAG TCC ATC ACG ATG CCA GTG CTA CGG 3’; 7B6 primer sense, 5’ CGC AGG CTG AAG AGG AG 3’; 7B6 primer antisense, 5’ GGC CAC AAG AGA ACA GAA C 3’; GAPDH primer sense, 5’ ATG GGG AAG GTG AAG GTC G 3’; GAPDH primer antisense, 5’ GGG GTC ATT GAT GGC AAC A 3’.

Control reactions with RNA used as template were also carried out to exclude the possibility of genomic contamination and were consistently negative. In addition, DNA was extracted from FRTL-5 cells and subjected to 37 cycles of PCR amplification using the NIS primers. PCR products of 1200 bp were amplified from genomic DNA, but the predicted 450 bp product could not be detected, further excluding the possibility of PCR artefacts that may arise from genomic DNA contamination.

**Oligonucleotide hybridization**

Product identification was confirmed by hybridization using internal oligonucleotide probes, as described
previously (Ajjan et al. 1996). In order to enhance the linearity of response of the radiographic film, preflashing was used throughout as described by Sambrook et al. (1989). The intensity of the bands was determined by densitometry, by the application of volume density analysis in the molecular analyst programme using the Biorad GS 690 scanning densitometer and the ratio of NIS/β-actin was subsequently calculated.

Iodide uptake

FRTL-5 cells were cultured in 12-well plates and deprived of TSH, when they reached 70% confluence for a period of 5 days. Cells were then treated with increasing concentrations of either bTSH or hTSH with or without the addition of cytokines. After 24–28 h incubation, iodide uptake in these cells was determined by the addition of 8 kBq of iodine-125 for a period of 1 h. Cells were then washed quickly with ice-cold PBS, solubilised with 1 ml 1 M sodium hydroxide, and radioactivity was counted using a gamma counter. All experiments were performed in duplicate cultures.

Results

NIS gene expression in FRTL-5 cells

The expression of GAPDH, 7B6 and β-actin mRNA was compared in TSH-deprived and TSH-stimulated cells using triplex RT-PCR. Expression of these genes was similar in eight samples studied on three occasions (data not shown). Therefore, only one house-keeping gene (β-actin) was used in the remainder of the study. Bovine TSH stimulated NIS gene expression in TSH-deprived FRTL-5 cells in a dose-dependent manner, reaching a peak at a concentration of 50 mU/l bTSH (Fig. 1). Human TSH had similar effects, but greater concentrations (10–10000 mU/l) were required (data not shown). The lower efficacy of hTSH is possibly due to altered glycosylation. TSH-induced NIS expression was evident after 6 h of TSH treatment, but not during the first 2 h (Fig. 2). TNFα inhibited TSH-stimulated NIS expression in a dose-dependent manner (Fig. 3), an effect that was detected with five different concentrations (0–1, 1, 10, 50 and 100 mU/l) of bTSH used (data not shown). The downregulation of TSH-stimulated NIS gene expression ranged from 65 to 75% when concentrations of 1000 U/ml TNFα were used (Fig. 4). TNFα also inhibited basal expression of NIS (Fig. 5). IL-1α inhibited both basal and TSH-stimulated NIS expression, again in a dose-dependent manner (Figs 3, 5), with an inhibition ranging from 65 to 80% when concentrations of 1000 U/ml IL-1α were used (Fig. 4). IFNγ also inhibited TSH-stimulated hNIS gene expression, but only at a concentration of 1000 U/ml, with an inhibition ranging from 40 to 65% (Fig. 4), whereas it had no effects at concentrations of 100 U/ml or less (Fig. 3). Cell culture experiments were repeated on two occasions, and PCR amplifications were performed two or three times with each set of cell culture. The mean interassay coefficient of variation, determined by analysing 22 samples on three occasions, was 19% (± 2.7).

Iodide uptake in FRTL-5 cells

Iodide uptake in FRTL-5 cells increased after bTSH treatment in a dose-dependent manner, with an increase clearly evident with concentrations of 10 mU/l (Fig. 1). Human TSH also increased iodide uptake in these cells, but again required greater concentrations to achieve effects similar to those of bTSH (data not shown). IL-1α, TNFα and IFNγ all inhibited iodide uptake in these cells at concentrations of 100 and 1000 U/ml, whereas little effect was detected with concentrations of 10 U/ml (Fig. 3).

Tissue samples

NIS expression was studied in Wistar rat thyroid, mammary gland, stomach, intestine, liver, fat, kidney and...
salivary gland tissue samples (Fig. 6). Except for the kidney, NIS mRNA was detected in all tissue samples studied, but with varying levels of expression. The greatest level of expression was found in the thyroid and stomach, followed by the mammary gland. Lower levels were detected in the intestine and adipose tissue from the orbit, epididymis, shoulder and abdomen. Very low levels were detected in the liver, and borderline expression was found in the salivary gland. Using 37 cycles of PCR amplification, NIS expression was clearly detected in the salivary gland, but not in kidney tissues (data not shown).

Discussion

In the present study, we have developed a semi-quantitative method, duplex RT-PCR, that overcomes the limitations of qualitative PCR. This can be applied to analyse small numbers of cells or small tissue samples, which would not be possible using Northern blot or RNase protection assays. Duplex RT-PCR was used throughout this study, with primers both for NIS and β-actin, a ubiquitously expressed housekeeping gene. This technique eliminates the artefacts that may arise from differences in first-strand cDNA synthesis between samples, and any variation in PCR conditions affects results obtained both with NIS and with β-actin. In addition, low numbers of cycles were used to demonstrate the abundance of the mRNA detected. A previous study has shown upregulation of β-actin gene expression after high-dose TSH treatment for an extended period of time (Phillips et al. 1989). In the present study, we included two additional house-keeping genes and showed that their expression was parallel to that of β-actin, ruling out...
TSH-mediated regulation of β-actin mRNA expression in our cell line.

Previous studies on FRTL-5 cells have shown that iodide uptake is dependent on the presence of TSH in the culture medium. Iodide uptake in these cells is almost completely abolished after 1 week of TSH deprivation (Weiss et al. 1984a), and is restored by the readdition of TSH. In the present study, NIS gene expression was constitutively expressed in TSH-deprived FRTL-5 cells, and was upregulated after TSH treatment, an effect detected after 6 h of stimulation, but not during the first 2 h. A recent study using Northern blot analysis has also shown similar effects of TSH regulation of NIS mRNA expression in FRTL-5 cells and demonstrated dependence on adenylate cyclase activation (Kogai et al. 1997). The readdition of TSH to TSH-deprived FRTL-5 cells results in increased iodide uptake after a latency period of 12–24 h, taking 60 h before cells reach full iodide uptake capacity (Weiss et al. 1984a). Therefore, it seems likely that this response is due, at least in part, to the induction of NIS gene expression, which is enhanced within 6 h of TSH treatment, taking a further 12–24 h for the appearance of biological activity. Although NIS mRNA levels were increased after βTSH stimulation from concentrations as low as 0·1 mU/l, no significant increase in iodide uptake was detected at concentrations less than 10 mU/l at 24–28 h. This is probably due to the time...
required for protein synthesis, as optimal iodide uptake in FRTL-5 cells is usually detected after 60 h of TSH stimulation (Weiss et al. 1984a).

The importance of NIS expression in iodide uptake was further emphasised in a recent study which showed that NIS mRNA is not expressed in thyroid carcinoma cell lines that have lost iodide uptake activity (Smanik et al. 1996). In contrast, the constitutive expression of NIS gene in TSH-deprived FRTL-5 cells may explain the findings of Kaminsky et al. (1994), who have demonstrated that membrane vesicles prepared from FRTL-5 cells maintained in TSH-free medium are able to accumulate iodide. It was proposed that NIS protein is present in TSH-deprived FRTL-5 in an inactive form, and is activated upon the readddition of TSH, through unknown mechanisms. Therefore, it is possible that downregulation of the NIS gene in FRTL-5, in the absence of TSH, is partly responsible for the decreased iodide uptake, and the symporter is present in these cells, but inactive because of the absence (or presence) of other unknown modulating factors regulated by TSH.

The role of cytokines in regulating thyroid function has been the focus of many studies over the past few years. It has been shown previously that TNFα inhibits FRTL-5 iodide uptake in vitro (Pang et al. 1993), whereas prolonged administration of TNFα to mice produced a dose-dependent decrease in concentrations of tri-iodothyronine (T3), thyroxine and recombinant T3 (Ozawa et al. 1988). Administration of TNFα to healthy volunteers induces changes in TSH and thyroid hormones resembling those found in non-thyroidal illness (NTI), suggesting involvement of TNFα in NTI, in patients with infections or malignancy, in whom production of TNFα is a feature (Van der Poll et al. 1990). In our study, TNFα inhibited both constitutive and TSH-stimulated NIS gene expression in vitro, indicating that the above effects of TNFα may be due, at least in part, to inhibition of NIS gene transcription. TNFα produced by the lymphocytic infiltrate in autoimmune thyroid disease may inhibit NIS mRNA expression. Hashimoto’s thyroiditis-derived lymphocytes produce TNFα (Del Prete et al. 1989), and thus it is possible that suppression of NIS gene expression by TNFα plays a part in hypothyroidism in the early stages of Hashimoto’s thyroiditis, before widespread tissue destruction becomes evident later in the disease process. A similar mechanism may partially counter the effect of thyroid stimulating antibodies (TSAb) in Graves’ disease, in which intrathyroidal TNFα also occurs (Zheng et al. 1992, Aust et al. 1996). This would contribute to the lack of correlation between the level of TSAb and the severity of clinical hyperthyroidism (Takata et al. 1980).

The effects of IL-1α on NIS gene expression mimicked those of TNFα, whereas IFNγ had an effect only when high concentrations of this cytokine were used. A good correlation between NIS gene expression and iodide uptake in FRTL-5 cells was detected, suggesting that cytokines affect iodide uptake in these cells by directly modulating NIS mRNA expression. Clinical considerations similar to those for TNFα would apply to the action of IL-1α and IFNγ on the NIS.

We have studied the tissue distribution of NIS and shown that iodide transporting tissues, including the stomach, mammary gland and thyroid, express high levels of the symporter. The intestine in rats also concentrates iodide, an activity that is maximal in the middle region of the small intestine (Pastan 1957). We have detected relatively low levels of NIS mRNA in the intestine, which is possibly due to the analysis of tissues containing both iodide transporting and non-transporting intestinal segments. Northern blotting has failed to detect NIS mRNA in rat intestine (Dai et al. 1996), emphasising the greater sensitivity of RT-PCR used in the current study.

The salivary glands typically transport iodide in many species; however, this is not the case in rats (Brown-Grant 1961, Wolff 1964). In the present study, only borderline levels of NIS mRNA were detected in the salivary glands. This probably explains earlier reports that have studied different rat species and shown that rat salivary glands are unable to concentrate iodide (Brown-Grant 1961, Wolff 1964).

An unexpected finding was the expression of NIS in organs that are believed not to transport iodide. Rat liver, which has been shown to lack NIS mRNA using Northern blotting (Dai et al. 1996), expressed the symporter. These contradictory results are probably due to the greater sensitivity of the PCR method we used, although only 27 cycles of PCR amplification were applied. NIS was also detected in adipose tissue excised from different sites, namely the epididymis, shoulder, abdomen and, most importantly, the orbit. It has been suggested recently that NIS is a novel autoantigen, as antibodies in a serum sample from a patient with Hashimoto’s thyroiditis inhibited iodide uptake in dog thyroid cell cultures (Raspé et al. 1995). In addition, recombinant rat NIS reacted with 84% of sera from patients with Graves’ disease (Endo et al. 1996b). More recently, four of 34 sera from patients with Hashimoto’s thyroiditis were shown to inhibit iodide uptake in Chinese hamster ovary cells stably expressing the rat NIS (Endo et al. 1996d). Furthermore, these sera reacted with a synthetic peptide corresponding to the sixth extracellular loop of NIS. The detection of NIS mRNA in orbital fat, together with the above findings, suggests that NIS is a candidate autoantigen involved in thyroid-associated ophthalmopathy, particularly in view of the greater incidence of the autoantibodies to the NIS in Graves’ disease compared with Hashimoto’s thyroiditis. However, NIS expression remains to be demonstrated in human retro-ocular tissue, and mRNA expression does not necessarily correlate with protein production. In addition, illegitimate transcription can sometimes produce artefactual signals (Chelly et al.
In summary, NIS gene expression in FRTL-5 cells is regulated by TSH and can be modulated by cytokines. The analysis of NIS gene tissue distribution in rats has shown that known iodide transporting tissues generally express high levels of NIS mRNA, and that this can also be detected, albeit at lower levels, in tissues that are believed not to transport iodide.

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