Posttranscriptional regulation of sodium-iodide symporter mRNA expression in the rat thyroid gland by acute iodide administration

Caroline Serrano-Nascimento, Jamile Calli-Silveira, and Maria Tereza Nunes

Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil

Submitted 22 May 2009; accepted in final form 14 January 2010

Serrano-Nascimento C, Calli-Silveira J, Nunes MT. Posttranscriptional regulation of sodium-iodide symporter mRNA expression in the rat thyroid gland by acute iodide administration. Am J Physiol Cell Physiol 298: C893–C899, 2010. First published January 27, 2010; doi:10.1152/ajpcell.00224.2009.—Iodide is an important regulator of thyroid activity. Its excess elicits the Wolff-Chaikoff effect, characterized by an acute suppression of thyroid hormone synthesis, which has been ascribed to serum TSH reduction or TGF-β increase and production of iodolipids in the thyroid. These alterations take hours/days to occur, contrasting with the promptness of Wolff-Chaikoff effect. We investigated whether acute iodide administration could trigger events that precede those changes, such as reduction of sodium-iodide symporter (NIS) mRNA abundance and adenylation, and if perchlorate treatment could counteract them. Rats subjected or not to methylmercaptimidazole treatment (0.03%) received NaI (2,000 μg/0.5 ml saline) or saline intraperitoneally and were killed 30 min up to 24 h later. Another set of animals was treated with iodide and perchlorate, in equimolar doses. NIS mRNA content was evaluated by Northern blotting and real-time PCR, and NIS mRNA poly(A) tail length were significantly reduced in all periods of iodide treatment. Perchlorate reversed these effects, indicating that iodide was the agent that triggered the modifications observed. Since the poly(A) tail length of mRNAs is directly associated with their stability and translation efficiency, we can assume that the rapid decay of NIS mRNA abundance observed was due to a reduction of its stability, a condition in which its translation could be impaired. Our data show for the first time that iodide regulates NIS mRNA expression at posttranscriptional level, providing a new mechanism by which iodide exerts its autoregulatory effect on thyroid.

NIS mRNA poly(A) tail; RACE-PAT; perchlorate

There is a growing body of evidence showing that trace elements, such as iron and selenium, modify the expression of proteins that are involved in their transport and metabolism by posttranscriptionally regulating the expression of the mRNAs that encode them. This posttranscriptional regulation mechanism rapidly changes gene expression patterns and occurs mainly at the transcript polyadenylation level, leading to alterations of the mRNA poly(A) tail length, which has been directly associated to transcript stability and translation efficiency (1–3, 34).

Iodine is known to acutely regulate the expression of the sodium-iodide symporter (NIS), a specific protein present in the basement membrane of thyroid cells and that mediates iodide uptake (8). This trace element is essential for thyroid hormone synthesis, although its excess causes a blockade of the thyroid function, which is known as the Wolff-Chaikoff effect (29, 49). This effect is rapid and transitory, and the mechanisms that underlie its initiation and the escape phenomenon are still not completely understood. It has been proposed that the escape effect is associated with the downregulation of NIS expression (mRNA and protein) and activity. These changes would decrease the intracellular iodide concentration, a condition in which the thyroid function blockade could not be sustained (6, 13, 48).

Even though the control of NIS mRNA abundance by iodide excess is mainly associated with a transcriptional effect, there is evidence suggesting that it might occur at a posttranscriptional level (13, 14, 43). In fact, the rapid onset and disappearance of the Wolff-Chaikoff effect strongly support the latter hypothesis. This study attempted to investigate that possibility by evaluating whether the acute iodide administration, from 30 min up to 24 h, could decrease the NIS mRNA abundance and poly(A) tail length, which would add a new insight into the molecular mechanisms involved in the control of NIS gene expression.

MATERIALS AND METHODS

Animals and Treatments

Male Wistar rats weighing 200–250 g were obtained from our own breeding colony and maintained on rat chow and tap water ad libitum. They were housed in a room kept at constant temperature (23 ± 1°C) and on a 12:12-h light-dark cycle (lights on at 0700) schedule. The animals were treated or not treated with 0.03% methylmercaptimidazole (MMI) (Sigma Chemical, St. Louis, MO) in drinking water, during 4 days. MMI inhibits the thyroperoxidase (TPO) activity, leading to a decrease of thyroid hormone synthesis and an increase of serum TSH concentration, which is known to upregulate NIS gene expression. Moreover, MMI blocks the production of iodolipids, which are supposed to mediate the inhibitory effect of the iodide excess on thyroid gland activity. Thus, its use is intended to highlight the effects of the iodide itself on the thyroid gland autoregulation process, and on the NIS mRNA expression.

Rats treated or not treated with MMI were assorted in two groups, one of which received iodide treatment (I) and the other received only the vehicle. MMI-treated rats received a single intraperitoneal injection of 2,000 μg NaI in 0.5 ml 0.9% NaCl (13) or vehicle (0.5 ml 0.9% NaCl) and were killed by decapitation 30 min to 24 h thereafter (I30 min, I1 h, I2 h, I12 h, and I24 h). Rats that were not treated with MMI were subjected to a similar schedule of iodide/vehicle treatment, except that they were killed 30 min or 24 h thereafter.

A subsequent study was carried out in rats treated or not treated with MMI that were 7) subjected to NaI, concomitantly with sodium perchlorate (2,000 μg of NaI or NaClO4 in 0.5 ml 0.9% NaCl) administration, and killed 30 min thereafter (I + P), and 2) treated for 30 min with NaI and then received perchlorate for an additional 30-min (I30 min + P30 min) period, after which they were killed.

The thyroid glands were rapidly excised for total RNA extraction, to investigate the abundance and poly(A) tail length of NIS mRNA, as described below. Blood samples were collected from the trunk for
evaluation of serum triiodothyronine (T₃), thyroxine (T₄), and TSH concentrations to check the efficiency of the MMI treatment.

The experimental protocol conformed with the ethical principles in animal research adopted by the Brazilian College of Animal Experimentation and was approved by the Institute of Biomedical Sciences/University of São Paulo-Ethical Committee for Animal Research (no. 75/2005).

Procedures

Evaluation of NIS mRNA abundance. The abundance of NIS transcript was examined by Northern blot analysis and real-time PCR. Thyroid total RNA extraction was performed by means of the acid guanidinium thiocyanate-phenol-chloroform extraction method according to a standard protocol (9, 27).

For Northern blot analysis of NIS mRNA abundance, 10 μg of denatured RNA were subjected to electrophoresis in 1.0% agarose gel, containing 2.2 M formaldehyde, in 1/100 MOPS, and blotted to a nylon membrane (GIBCO-BRL, Rockville, MD) by neutral capillary transfer. Subsequently, the membrane was probed with a 32P-labeled rat NIS cDNA, for 16 h at 42°C. The membrane was washed under highly stringent conditions and subjected to autoradiography, and blots were quantified by means of ImageJ software (National Institutes of Health, Bethesda, MD). All blots were stripped and rehybridized with a 32P-labeled β-actin cDNA, to correct the variability in RNA loading (43).

Real-time PCR assay was used to evaluate NIS mRNA content in thyroids of rats that were not treated with MMI. The integrity of RNA was confirmed by inspecting the electrophoretic pattern of 28S and 18S ribosomal RNA bands in ethidium bromide-stained agarose gel (1%). Three micrograms of total RNA were used in a reaction containing oligo(dT) (100 μg/ml); 10 mM of each dNTP; 5X first-strand buffer, 0.01 M of DTT, and 1 μl of 200 U/μl of Moloney murine leukemia virus (MMLV) reverse transcriptase (Invitrogen Life Technologies, Carlsbad, CA). Reverse transcription was performed at 70°C for 10 min, 47°C for 60 min, and 10 min at 95°C. Then, 1 μl of each reverse transcription reaction product was diluted in a reaction buffer containing 5 μl of Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen Life Technologies); 0.5 μl of primers (forward and reverse primers for NIS and β-actin, which was used as endogenous control) in a final volume of 10 μl per sample. All primers used were selected and optimized for real-time PCR analysis. The reaction conditions consisted of two steps at 50°C for 2 min and 95°C for 5 min, followed by 40 cycles of three steps: 20 s at 95°C (denaturation), 60 s at 60°C (annealing), and 20 s at 72°C (extension). The threshold

Table 1. Serum T₃, T₄, and TSH concentration in rats treated or not with MMI and subjected or not to acute NaI treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>Total T₃ Serum Concentration, ng/ml</th>
<th>Total T₄ Serum Concentration, ng/ml</th>
<th>TSH Serum Concentration, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.41 ± 0.07</td>
<td>172.83 ± 2.30</td>
<td>2.38 ± 0.46</td>
</tr>
<tr>
<td>Iodide</td>
<td>0.38 ± 0.05</td>
<td>169.25 ± 3.10</td>
<td>2.29 ± 0.32</td>
</tr>
<tr>
<td>Control (MMI)</td>
<td>0.08 ± 0.01*</td>
<td>76.38 ± 4.83*</td>
<td>7.69 ± 1.00*</td>
</tr>
<tr>
<td>Iodide (MMI)</td>
<td>0.08 ± 0.02*</td>
<td>84.53 ± 5.35*</td>
<td>7.54 ± 0.53*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7 per group. T₃, triiodothyronine; T₄, thyroxine; MMI, methylmercaptoimidazole. *P < 0.001 vs. control and iodide groups.

Fig. 1. Sodium-iodide symporter (NIS) mRNA abundance is reduced by iodide excess in rats previously treated with methylmercaptoimidazole (MMI). Northern blot analysis of NIS mRNA abundance was performed in thyroids of rats treated with NaI for 30 min (A), 1 h (B), 2 h (C), 12 h (D), and 24 h (E). Typical autoradiographs of NIS and β-actin mRNAs of one experiment are shown at the top of each diagram, and the quantitative representation obtained by densitometric analysis of NIS and β-actin transcripts hybridization ratio is shown at the bottom in arbitrary units. I, iodide treatment group; C, control group. Results are expressed as means ± SE of 3 independent experiments. At least three animals per group were used in each experiment. *P < 0.05, **P < 0.01 vs. C (MMI) (unpaired two-tailed Student’s t-test).
iodide treatment did not cause alterations in any of those parameters.

Effect of NaI Administration on NIS mRNA Abundance and Polyadenylation

The NaI treatment (from 30 min to 24 h) significantly decreased the NIS mRNA abundance in MMI-treated rats, in comparison to the respective control group values, as illustrated in Fig. 1, A–E. A similar effect was observed when rats not treated with MMI were subjected to NaI excess for 30 min and 24 h, as demonstrated in Fig. 2, A and B.

The NIS mRNA poly(A) tail length in rats subjected or not to MMI (Figs. 3 and 4, respectively) was also decreased by NaI treatment, for all periods studied, as revealed by the analysis of the smearing pattern of PCR products obtained by RACE-PAT. This analysis showed a smaller number of base pairs in the NIS transcript poly(A) tail in iodide-treated rats than their respective control groups. The largest alteration of poly(A) tail length was observed after 30 min of NaI administration, a period in which rats subjected or not to MMI treatment showed a reduction of approximately 65 and 55 adenine residues of the NIS mRNA poly(A) tail, respectively, when compared with the control group (P < 0.001).

Effect of the NaClO₄ Administration on NIS mRNA Abundance and Polyadenylation

Considering that the findings described above were most pronounced 30 min after NaI administration, we have selected this period of time to carry out this particular analysis.

The data on NIS mRNA abundance and poly(A) tail length obtained from thyroids of MMI-treated rats that received sodium perchlorate concomitantly with (I + P) or 30 min after (I₃₀min + P₃₀min) NaI administration are illustrated in Figs. 5 and 6, respectively. The simultaneous administration of perchlorate and iodide prevented the reduction of NIS mRNA content (Fig. 5) and poly(A) tail length (Fig. 6) observed when rats were treated only with NaI. When sodium perchlorate was administered 30 min after NaI treatment, the decrease of NIS transcript and poly(A) tail length induced by NaI was reversed. Similar results were observed in rats that were not previously treated with MMI, but received perchlorate concomitantly with (I + P) or 30 min after (I₃₀min + P₃₀min) NaI administration, as pointed out in Figs. 7 and 8.

DISCUSSION

Iodide is a key element for the thyroid gland physiology. Its entry in the thyroid gland is an essential step for thyroid

![Graph A](attachment:graph_a.png)

**A** NIS mRNA abundance is reduced by iodide excess in rats not previously treated with MMI. Real-time PCR analysis of NIS mRNA abundance was performed in thyroid of rats treated with NaI for 30 min (A) or 24 h (B). NIS mRNA abundance levels were normalized to β-actin mRNA content. The quantitative representation is shown in arbitrary units. Results are expressed as means ± SE of 3 independent experiments. At least three animals per group were used in each experiment. *P < 0.05, **P < 0.001 vs. C (unpaired two-tailed Student’s t-test).
hormone synthesis and depends on NIS activity, which, in turn, is modulated by the intraglandular iodide content. Indeed, intracellular iodide excess was shown to reduce NIS expression, at both mRNA and protein levels (13, 14, 43, 44). This specialized intrinsic autoregulatory mechanism ensures adequate iodide uptake and at the same time protects the gland from the deleterious effects of high doses of iodide (21, 48).

The data presented herein are in accordance with the above considerations, since the treatment of rats with a high iodide dose induced a reduction of NIS mRNA content. In addition, they add a new perspective for that regulatory process, considering the promptness of the iodide effects (30 min) that strongly suggests the involvement of posttranscriptional mechanisms in the regulation of NIS expression by iodide excess. This assumption is reinforced by the fact that NIS mRNA reduction was detected even in the presence of high serum TSH concentration, secondary to previous MMI treatment, a situation in which an increase of NIS mRNA abundance is expected to occur (26, 33, 45).

The rapid decrease of NIS mRNA abundance (30 min) induced by iodide administration led us to speculate that the transcript stability could be reduced in this experimental condition. Considering that differences in the mRNA poly(A) tail length have been previously reported to affect its stability (10, 30, 35, 38), polyadenylation tests were performed, according to the rapid amplification of cDNA ends-polyadenylation technique (RACE-PAT). Our results revealed that NIS transcript poly(A) tail length was reduced in thyroids of rats treated from 30 min to 24 h with iodide. These findings reinforce previous results obtained in our laboratory, which showed a higher electrophoretic mobility of NIS mRNA of rats subjected to the same treatment schedule (41).

In fact, we cannot assure that the reduction of poly(A) tail length promoted by iodide excess is specific to the mRNA encoding NIS. However, studies performed in our laboratory with pendrin mRNA have provided evidence that the poly(A) tail length of this transcript is not altered by iodide excess treatment, which indicates that iodide differentially regulates the expression of genes involved in its metabolism on thyroid cell (Calil-Silveira J, Serrano-Nascimento C, and Nunes MT, unpublished observations).

The polyadenylation is one of the most important steps of the posttranscriptional control of gene expression (18, 46). The addition of adenine residues to the transcript’s 3'-untranslated region confers stability to it, since the poly(A)-binding protein binds to the poly(A) tail, protecting it from the exoribonuclease attack (4, 5, 7, 36, 37, 47) and also allows a better attachment of the transcript to the cap region, generating higher translation efficiency (22).

Although we have not addressed this question directly, it is likely that the shortened NIS mRNA poly(A) tail length of the
iodide-treated rats led this transcript to be more susceptible to RNase attack. This could contribute to a change of its half-life, and a rapid reduction of its content. It can also be expected that the efficiency of the NIS mRNA translation would be impaired in this condition, as pointed out before (30). Even though these findings point to a posttranscriptional control of NIS mRNA expression by iodide, they do not exclude the possibility that longer periods of iodide exposure could also affect the NIS gene transcription rate, as already described (13, 14, 24).

To confirm that these effects were elicited by intracellular iodide excess, we have performed studies in which perchlorate was administered concomitantly with, or 30 min after, the rats have been treated for 30 min with NaI. These strategies aimed, respectively, to prevent iodide entry in the thyroid gland, considering that perchlorate competes with iodide for its site on NIS (11, 50, 51), and also to induce the release of iodide not incorporated to thyroglobulin, especially in the animals previously treated with MMI, a condition in which iodide organification is impaired (16).

The prevention of the NaI-induced reduction of NIS mRNA abundance and adenylation, when perchlorate and iodide were concomitantly administered, indicates that iodide entry in the thyroid gland is essential to trigger the effects reported. This is reinforced by the fact that the perchlorate treatment, which is known to facilitate the discharge of free iodide from the thyroid gland, reversed the reduction of NIS transcript abundance and polyadenylation induced by previous administration of NaI, when it was administered 30 min after iodide injection. These results strongly point to iodide as the agent that triggers the modifications of the NIS mRNA described in this study.

Another fact to be highlighted is that the inhibitory effect promoted by iodide excess on the thyroid gland has been ascribed to the iodolipids production by TPO (17, 20). In fact, we cannot exclude the possibility of a role of iodolipid production in the regulation of NIS gene expression, because a higher reduction of NIS mRNA content was observed after iodide excess administration in rats that did not undergo perchlorate treatment.

Fig. 4. NIS mRNA poly(A) tail length, analyzed by RACE-PAT, is reduced in rats not treated with MMI, subjected to iodide excess for 30 min (A) or 24 h (B). Left: smearing pattern of PCR products of control and iodide-treated rat samples, respectively, in ethidium bromide-stained 2.5% agarose gel. The horizontal gray line delimits the longest amplified fragments generated in the samples of iodide groups. L, 100-bp DNA ladder. Right: the maximal sizes of the amplicons, corresponding to the top of the smear of control (black bars) and iodide-treated (white bars) groups, are shown in base pairs. Results are expressed as means ± SE of 3 independent experiments. At least three animals per group were used in each experiment. *p < 0.05, ***p < 0.001 vs. C (unpaired two-tailed Student’s t-test).

Fig. 5. Perchlorate (P) treatment prevented the effects of iodide on NIS mRNA abundance in rats previously treated with MMI. Northern blot analysis of NIS mRNA abundance was performed in thyroid of C (MMI), I30 min (MMI), I30 min + P30 min (MMI), and I + P (MMI) groups. Typical autoradiographs of NIS and β-actin mRNAs of one experiment are shown at top, and the quantitative representation obtained by densitometric analysis of NIS and β-actin transcripts hybridization ratio is shown at bottom, in arbitrary units. Results are expressed as means ± SE of 3 independent experiments. At least three animals per group were used in each experiment. **p < 0.01 vs. C (MMI); #p < 0.05 vs. I30 min + P30 min (MMI); ●●p < 0.01 vs. I + P (MMI) (one-way analysis of variance with Student-Newman-Keuls as posthoc test).

Fig. 6. Perchlorate treatment prevented the effects of iodide excess on NIS mRNA poly(A) tail length in rats previously treated with MMI. Left: smearing pattern of PCR products of control and iodide-treated rat samples, respectively, in ethidium bromide-stained 2.5% agarose gel. The horizontal gray line delimits the longest amplified fragments generated in the samples of iodide groups. L, 100 bp. Right: the maximal sizes of the amplicons, corresponding to the top of the smear of control (MMI) (black bar), I30 min (MMI) (white bar), I30 min + P30 min (MMI) (gray bar), and I + P (MMI) (gray hachured bar) groups are shown in base pairs. Results are expressed as means ± SE of 3 independent experiments. At least three animals per group were used in each experiment. **p < 0.001 vs. C (MMI); #p < 0.01 vs. I30 min + P30 min (MMI); ●●●p < 0.001 vs. I + P (MMI) (one-way analysis of variance with Student-Newman-Keuls as posthoc test).
treatment with MMI. However, in rats previously treated with MMI, which is known to block TPO activity (15, 31, 42) and, as a consequence, the production of iodolipids, the effect of NaI excess on NIS mRNA abundance and polyadenylation was preserved, which strengthens our hypothesis that iodide itself is the key element for eliciting the effects reported. This assumption is also reinforced by the studies performed by Leoni et al. (24), which showed that PCC13 cells, under iodide and MMI treatment, presented a reduction of NIS gene expression, indicating that this anion per se is capable of regulating the NIS expression.

The data presented herein increase the body of evidence that demonstrates that trace elements can regulate, at a post-transcriptional level, genes that encode for proteins related to their transport or metabolism, such as iron, selenium, and calcium, which are known to regulate the mRNA abundance and stability of transferrin receptor, cytosolic glutathione peroxidase, and parathyroid hormone, respectively. The mechanisms that underlie this regulation involve the interaction of these trace elements with proteins bound to mRNA untranslated regions (1, 2, 3, 23, 28, 32, 34, 40), and it is possible that iodide might alter NIS mRNA abundance by similar mechanisms.

In conclusion, our results demonstrate, for the first time, that acute iodide administration reduces NIS mRNA content through a post-transcriptional mechanism which involves the shortening of NIS mRNA poly(A) tail length, an effect that can be demonstrated as soon as 30 min after iodide administration. This might account for the reduction of NIS expression observed in similar protocols of study, as well as for the escape of the Wolff-Chaikoff effect, which is installed by the administration of high doses of iodide. The present data indicate that iodide plays an essential role in the thyroid gland homeostasis, by promptly regulating its own concentration within the gland, and thus assuring adequate iodide uptake for thyroid hormone synthesis, and protecting the thyroid from the detrimental effects of its excess. Thus, by combining both transcriptional and post-transcriptional mechanisms, iodide may provide a mechanism for tight control of NIS gene expression regulation in the thyroid gland.

Fig. 7. Perchlorate treatment prevented the effects of iodide excess on NIS mRNA abundance in rats not previously treated with MMI. Real-time PCR analysis of NIS mRNA abundance was performed in thyroid of C, I10 min, I30 min + P30 min, and I + P groups. NIS mRNA abundance levels were normalized to β-actin mRNA content. The quantitative representation is shown in arbitrary units. Three animals per group were used in each experiment. ***P < 0.001 vs C; #P < 0.05 vs I10 min + P30 min; ●●P < 0.01 vs I + P (one-way analysis of variance with Student-Newman-Keuls as posthoc test).

Fig. 8. Perchlorate treatment prevented the effects of iodide excess on NIS mRNA poly(A) tail length in rats not previously treated with MMI. Left: smearing pattern of PCR products of C, I10 min, I30 min + P30 min, and I + P rat samples, respectively, in ethidium bromide-stained 2.5% agarose gel. The horizontal gray line delimits the longest amplified fragments generated in the samples of iodide groups. L, 100 bp. Right: the maximal sizes of the amplicons, corresponding to the top of the smear of C (black bar), I10 min (white bar), I30 min + P30 min (gray bar), and I + P (gray hachured bar) groups, are shown in base pairs. Results are expressed as means ± SE of 3 independent experiments. At least three animals per group were used in each experiment. ***P < 0.001 vs C; ###P < 0.001 vs I10 min + P30 min; ●●P < 0.01 vs I + P (one-way analysis of variance with Student-Newman-Keuls as posthoc test).

ACKNOWLEDGMENTS

The authors are grateful to Leonice L. Poyares for excellent technical assistance and to Dr. Luiz R. G. Britto for reviewing the manuscript.

GRANTS

This work was supported by a fellowship from the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP; 06/52829-5) to C. Serrano-Nascimento. J. Calil-Silveira and M. T. Nunes are the recipients of fellowships from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

DISCLOSURES

No conflicts of interest are declared by the authors.

REFERENCES

IODIDE ACUTELY REGULATES NIS mRNA EXPRESSION


