Minimal oxidative load: a prerequisite for thyroid cell function

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Abstract

In addition to reactive oxygen species (ROS) produced by mitochondria during aerobic respiration, thyrocytes are continuously producing H_2O_2 , a key element for hormonogenesis. Because nothing is known about ROS implication in normal non-stimulated cells, we studied their possible involvement in thyrocytes incubated with a potent antioxidant, *N*-acetylcysteine (NAC). NAC, which blocked the production of intracellular ROS, also decreased dual oxidases, thyroperoxidase, pendrin, and thyroglobulin protein and/or gene expression. By contrast, Na^+/I^- symporter mRNA

expression was unaffected. Among antioxidant systems, peroxiredoxin (PRDX) five expression was reduced by NAC, whereas peroxiredoxin three increased and catalase remained unchanged. *In vivo*, the expression of both dual oxidases and peroxiredoxin five proteins was also decreased by NAC. In conclusion, when intracellular ROS levels drop below a basal threshold, the expression of proteins involved in thyroid cell function is hampered. This suggests that keeping ROS at a minimal level is required for safeguarding thyrocyte function.

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Introduction

Reactive species include both reactive oxygen species (ROS) and reactive nitrogen species (RNS). In all cell types, ATP generation by mitochondrial aerobic respiration generates ROS as by-products of oxidative phosphorylations. In physiological conditions, ROS in excess must be constantly detoxified by antioxidant systems to avoid cell damage. In thyrocytes, H_2O_2 is a ROS that is required for hormonogenesis. It is produced from dual oxidases (DUOX1/2) (Dupuy *et al.* 1999, De Deken *et al.* 2002) at the apical pole of the cell, where it oxidizes iodide (actively transported across the basal membrane by Na^+/I^- symporter (NIS, product of SLC5A5) (Dai *et al.* 1996)) into iodine in a reaction catalyzed by thyroperoxidase (TPO). Iodine is then incorporated into tyrosine residues of thyroglobulin (TG), again by TPO. Hence, H_2O_2 and free radicals are continuously produced in thyroid cells, even in physiological conditions (Denef *et al.* 1996, De Deken *et al.* 2002, Schweizer *et al.* 2008). Obviously, when ROS are over-produced in pathological situations, they may exert a wide range of actions, usually deleterious (Adler *et al.* 1999, Blokhina *et al.* 2003). To preserve cell integrity, several protective systems against ROS, such as peroxiredoxins (PRDX), catalase, and glutathione peroxidases are heavily expressed and active in thyrocytes. Thus, PRDX5 and glutathione peroxidase are increased in goitrous thyroids, both in human and rodents (Mano *et al.* 1997, Mutaku *et al.* 2002, Gerard *et al.* 2005, Poncin *et al.* 2008a). Cells can rely on various antioxidant tools to maintain the oxidative stress

(OS) in strict limits to avoid OS becoming eventually harmful. Among them, selenium, which is required for glutathione peroxidase activity, has been shown to exert protective effects in various situations where OS is elevated, while its defect is deleterious (Contempre *et al.* 1993, 2004, Schomburg & Kohrle 2008, Schweizer *et al.* 2008).

Up to now, nothing has been reported about the physiological implication of ROS in normal resting thyrocytes. To answer this question, we analyzed the *in vitro* and *in vivo* expression of proteins involved in thyroid cell function in the presence of *N*-acetylcysteine (NAC), an agent supposed to deprive cells from intracellular ROS, because of potent antioxidant properties. We also investigated the regulation of antioxidant systems when the cell oxidative load is reduced.

Materials and Methods

Cell cultures

PCCL3 cells (a continuous line of non-transformed rat thyroid follicular cells, Fusco *et al.* 1987) were a gift from Dr F Miot (Université Libre de Bruxelles, IRIBHN, Brussels, Belgium), and human thyroids from multinodular goiters were obtained at surgery after patients gave their informed consent. The study was performed after approval from the ethical committee had been received. Cells were cultured as previously reported (Poncin *et al.* 2008b). NAC (5, 2.5, 1, or 0.5 mM; Sigma–Aldrich) was added for 3 days in the cell medium containing 0.5% newborn calf serum and 1 mU/ml TSH. All experiments were repeated at least twice.

ROS production

Thyrocytes were incubated in multichamber glass slides in appropriate medium. ROS production was measured using a fluorescent dye, 2', 7' dichlorofluorescein diacetate (DCFH-DA, Molecular Probes, Paisley, UK). Phosphate buffer saline (PBS, pH 7.4)-washed thyroid cells were incubated in Krebs–Ringer–HEPES (KRH) medium (pH 7.4) containing DCFH-DA (25 µM) at 37 °C for 1 h. The excess of dye was removed by two washes with PBS. Cells were stained with Hoechst for 20 min and rinsed in PBS. Cover slides were mounted in fluorescent mounting medium (DakoCytomation, Carpinteria, CA, USA) for microscopic observation. ROS production was visualized on a fluorescent microscope equipped with a digital camera (Zeiss, Zaventem, Belgium).

Viability assay

Cell viability was assessed using the Alamar blue assay (Biosource International, Camarillo, CA, USA), as previously described (Gerard *et al.* 2006).

Apoptosis detection

Caspase activity was measured by using a CaspACE FITC-VAD-fmk *in situ* marker (Promega) that binds activated caspases, according to the manufacturer's instructions. Briefly, cells were incubated with 20 µM FITC-VAD-fmk at 37 °C for 20 min. Cells were then washed twice with PBS, dyed with Hoechst for 20 min, and rinsed in PBS. Cells were fixed in 10% buffered formalin for 30 min and rinsed with PBS. Cover slides were mounted in fluorescent mounting medium for microscopic observation. Cells treated with staurosporin (5 µM, Sigma) were used as positive controls.

Nitrite assay

Nitrite accumulation in the medium of human thyrocytes was measured by the Griess reaction using a commercially available kit (Promega).

Quantitative PCR

For each condition, cells from six individual wells were suspended in TriPure isolation reagent (Roche Diagnostics GmbH). Total RNA purification and reverse transcription were performed as previously described (Gerard *et al.* 2008). Quantitative PCR was performed in an iCycler apparatus (IQ5, Bio-Rad). cDNAs (2 µl) were mixed with 500 nM of each selected primers (Table 1) and SYBR green reaction mix (Bio-Rad) in a final volume of 25 µl. Reactions were performed as follows: 95 °C/1.5 min, followed by 40 cycles of 95 °C/15 s, annealing temperature (Table 1)/45 s, and 81 °C/15 s. Amplification levels were normalized to those of β-actin. All melting curves were analyzed for each PCR to avoid genomic DNA amplification.

Western blotting

Thyrocytes were suspended in Laemmli buffer (50 mM Tris–HCl, pH 6.8, 2% SDS, 10% glycerol), containing a protease inhibitor cocktail (Sigma), and were sonicated during 30 s. Protein concentration was determined using a BCA protein assay kit (Pierce, Rockfort, IL, USA). DUOXs (antibody provided by F. Miot, IRIBHN), TPO (antibody provided by J. Ruf, Université de la Méditerranée, Marseille, France), PRDX5 (antibody provided by B. Knoops, UCL, Louvain La Neuve), and β-actin (Sigma) western blottings were performed as previously described (Gerard *et al.* 2006). Membranes were blocked for 1 h at room temperature (RT) in PBS (pH 7.4), 5% non-fat dry milk, 0.1% Tween, and incubated overnight at 4 °C with the primary antibody at a dilution of 1/4000 (DUOXs, TPO), 1/10 000 (PRDX5), or 1/2000 (β-actin). Membranes were incubated for 1 h at RT with EnVision (1/200, DakoCytomation) peroxidase-labeled secondary antibody, and visualized with enhanced chemiluminescence (SuperSignal West Pico, Pierce) on CLXposure TM films (Pierce). Western blots were scanned and quantified by densitometry using the NIH Scion Image Analysis Software (National Institutes of Health, Bethesda, MA, USA). Densitometric values were normalized to β-actin expression.

Table 1 Forward and reverse primers and annealing temperatures

	Primer forward	Primer reverse	Annealing T (°C)
Actin	5'-CATCCTGCGTCTGGACCT-3'	5'-AGGAGGAGCAATGATCTTGAT-3'	62
r DUOX1/2	5'-GTGGCTGGAGGGAGCCAT-3'	5'-CCGTGAACAGACTCCTGT-3'	60
r TPO	5'-CAGGTTTTGGTGAGAA-3'	5'-CTGCACACTCATTAACATCTT-3'	58
r NIS	5'-GCGCTGCGACTCTCCCACTGAC-3'	5'-GGCGGTAGAAGATCGGCAAGAAGA-3'	60
r Pendrin	5'-CATCAAGACACATCTCCGTTGGCCCT-3'	5'-GGTACTTCCGTACCACTGGGC-3'	60
r TG	5'-GCAGAAACACCACATCACTGGAGC-3'	5'-TGGCACTGGGGACTCTGGACTTGAC-3'	59
h DUOX1/2	5'-GTGGCTGGCTGACATCAT-3'	5'-TGAGGGAGTTGAAGAA-3'	58
h TPO	5'-CACGATGCAGAGAAACCTCAA-3'	5'-ATAGACTGGAGGGAGCCAT-3'	60
h NIS	5'-ACCGCGCCCCACCTCTTTCTTATT-3'	5'-CCCCCTCCTGATTCTGGTTGTTG-3'	62
h Pendrin	5'-TGGAACATCAAGACATATCTCAGTTG-3'	5'-TGCTGCTGGATACGAGAAAGTG-3'	60
h TG	5'-CGCTGGCGGCTCAGTCTACCTT-3'	5'-AGCAGTTTCTGCGTGGGAG-3'	60

PRDX5 immunofluorescence

Thyrocytes were cultured in multichamber glass slides in appropriate medium. They were fixed for 30 min in 4% paraformaldehyde, rinsed once with PBS, permeabilized for 15 min in a PBS–Triton 1% solution at RT, and washed with PBS supplemented with 1% BSA (PBS–BSA). Cells were then incubated for 1 h with PRDX5 primary antibody (1/75) at RT. After being washed in PBS, a FITC-conjugated secondary antibody was added for 1 h at RT at a dilution of 1/30 (anti-rabbit; DakoCytomation). Cover slides were mounted in fluorescent-mounting medium for microscopic observation.

Animals and treatments

NMRI mice of 2-months-old received a standard diet. Animals were given i.p. injected of saline solution of NAC (100 mg/kg per day) for 4 days. Mice were housed and handled according to Belgian regulation of Laboratory Animal Welfare.

Preparation of tissue samples for microscopy

Five animals of each group (control and NAC) were anesthetized with pentothal and thyroid lobes were dissected. One thyroid lobe was fixed in paraformaldehyde (4% in PBS) for 24 h and embedded in paraffin. Thick sections (5 µm) were used for PRDX5 immunohistochemistry. The second lobe was frozen and cryostat sections (5 µm) were used for DUOXs immunohistochemistry.

DUOXs and PRDX5 immunohistochemistry

Tissue sections were washed with PBS–BSA and incubated at RT for 30 min with normal goat serum (1/50, Vector Labs, Burlingame, CA, USA) in PBS–BSA. Slides were then incubated at RT for 1 h with DUOXs (1/75) or PRDX5 (1/250) primary antibodies. The antibody was detected using Envision (DakoCytomation) or ABC kit (Vector labs) for DUOXs and PRDX5 detection respectively. The peroxidase activity was revealed using AEC (DakoCytomation) as substrate. Sections were counterstained with Mayer's hematoxylin.

Data analysis and statistics

Data were expressed as mean $\pm$ S.E.M., $n=6$ for all assays. All experiments were repeated at least twice. Statistical analyses were performed using ANOVA followed by Tukey–Kramer multiple comparison test (GraphPad InStat, San Diego, CA, USA), or by unpaired *t*-test. $P<0.05$ was considered as statistically significant.

Results

NAC reduces intracellular ROS production without inducing apoptosis or affecting cell viability

In human thyrocytes, ROS, as detected by DCFH-DA fluorescence (Fig. 1A), were mainly observed in cytoplasm

granules. NAC (1 mM)-treated cells showed a marked decrease in fluorescence. Identical results were obtained in PCCL3 cells (data not shown).

In human thyrocytes, nitrite levels (Fig. 1B, left panel), the stable end product of NO generation, were low in media from control cells and slightly increased in NAC (1 mM)-treated cells (1.6-fold, $P<0.05$). *Nos2* mRNA expression was not influenced by NAC (Fig. 1B, right panel).

In staurosporin-treated cells, used as positive control for apoptosis, all nuclei were labeled with CaspACE FITC–VAD–fink marker (Fig. 1C). By contrast, apoptosis was detected neither in control, nor in NAC (1 mM)-treated cells. Cell viability was therefore unaffected by NAC (Fig. 1D).

NAC reduces the expression of proteins involved in thyroid cell function

In human thyrocytes, DUOXs and TPO proteins were detected by Western blot (Table 2). NAC at concentrations ranging from 0.5 to 5 mM strongly decreased the expression of both proteins. As no difference was observed from one concentration to another, 1 mM was selected as reference concentration in all experiments. NAC also decreased *Duoxs* and *Tpo* mRNA expressions, both in human and PCCL3 cells (Table 3). By contrast, NAC had no effect on *Nis* mRNA expression. *Tg* and *pendrin* mRNAs were also downregulated in PCCL3 cells, but not in human cells. Thus, except NIS, all thyroid differentiation genes studied in this work were downregulated in ROS-deprived cells.

NAC differentially affects the expression of PRDX5 and PRDX3

Both in human and PCCL3 cells, NAC decreased PRDX5 protein expression by 2.6-folds ($P<0.05$, Fig. 2A). The analysis of PRDX5 protein expression by immunofluorescence (Fig. 2B) showed a cytoplasmic and granular pattern, suggesting a mitochondrial localization of the protein (Bannmeyer *et al.* 2005). By contrast, NAC induced a twofold increase in PRDX3 protein expression ($P<0.05$, Fig. 2C). Catalase protein expression remained unchanged (Fig. 2D).

NAC decreases DUOXs and PRDX5 expressions in mouse thyroids

In mouse thyroids, DUOXs was detected at the apical pole of thyrocytes (Fig. 3A). In NAC-treated mice, DUOXs immunostaining was more faint and detected in few cells (Fig. 3B). A PRDX5 signal (Fig. 3C) was present in the cytosol and in nuclei, as previously reported (Poncin *et al.* 2008a). In NAC-treated mice (Fig. 3D), the signal was strongly reduced, which is in accordance with the above reported *in vitro* observations.

Figure 1 NAC reduces intracellular ROS production without inducing apoptosis or affecting cell viability. ROS production was assessed in control- and NAC-treated cells using DCFH-DA fluorescence (A, upper panel). Nuclei were stained with Hoechst (A, lower panel). Scale bars, 20 µm. Nitrite accumulation in the culture medium of human thyrocytes (B, left panel) was assessed using Griess reaction. Results are expressed as mean $\pm$ S.E.M. of six ($n=6$) individual wells of one representative experiment. * $P<0.05$ versus control cells. *Nos2* mRNA expression in human thyrocytes was analyzed by qPCR (B, right panel). Values, adjusted to the β -actin signal, are expressed as mean $\pm$ S.E.M. of one representative experiment ($n=6$). Apoptosis was detected using a CaspACE FITC-VAD-fmk *in situ* marker (C) in staurosporin (5 µM)-treated cells used as positive control, in control cells, and in NAC-treated cells. Nuclei were stained with Hoechst (lower panel). Scale bars, 25 µm. Cell viability was assessed using Alamar Blue assay (D). Full colour version of this figure available via <http://dx.doi.org/10.1677/JOE-08-0470>.

Discussion

This study shows that PCCL3 and human thyroid cells produce a given amount of intracellular ROS in basal conditions, likely as a result of physiological aerobic metabolism. But thyrocytes are somewhat unique in that they produce H₂O₂ that, along with iodine, is a key element for TH synthesis. Thus, ROS in the thyroid are definitively more than just by-products of aerobic respiration. They are in fact intrinsically involved in endocrine function. H₂O₂ is produced outside the cell by NADPH oxidases, DUOXs localized at the apical pole of the thyrocyte (De Deken *et al.* 2002). The hormone synthesis then occurs in the follicular lumen, close to the apical pole. Until now, H₂O₂ has always been detected in culture media (Bjorkman & Ekholm 1992, Fortemaion *et al.* 2005), never inside the cell. Here, we show for the first time that ROS are physiologically produced into cells without provoking significant cell damage, despite the fact that basal DNA damage in thyrocytes is higher than in

other tissues (Krohn *et al.* 2007, Maier *et al.* 2007). H₂O₂ is likely one of these ROS as the DCFH-DA probe mainly reacts with H₂O₂. Besides DUOXs produced H₂O₂, other possible sources of intracellular ROS include mitochondria and peroxisomes (Bedard & Krause 2007). It is likely that ROS other than H₂O₂, such as hydroxyl radicals and anion superoxide, are produced in physiological conditions, but

Table 2 DUOXs and TPO protein expressions in human thyrocytes. DUOXs and TPO protein expressions in human thyrocytes were analyzed by western blot

	Human	
	Control	NAC 1 mM
DUOXs	2.39 $\pm$ 0.38	1.17 $\pm$ 0.38*
TPO	3.69 $\pm$ 0.72	0.98 $\pm$ 0.82*

Values, normalized to β -actin, are expressed as mean $\pm$ S.E.M. of one representative experiment ($n=6$). * $P<0.05$ versus control cells.

Table 3 Relative mRNA expression of thyroid genes. *Duoxs*, *Tpo*, *Nis*, *Tg*, and *pendrin* mRNA levels were measured by qRT-PCR in human thyrocytes and PCCL3 cells

	Human		PCCL3	
	Control	NAC 1 mM	Control	NAC 1 mM
<i>Duoxs</i>	1.61 ± 0.44	0.93 ± 0.27*	1.09 ± 0.13	0.62 ± 0.14*
<i>Tpo</i>	0.72 ± 0.11	0.15 ± 0.05*	0.81 ± 0.19	0.34 ± 0.08*
<i>Nis</i>	1.61 ± 0.53	1.59 ± 0.38	0.89 ± 0.17	0.79 ± 0.14
<i>Tg</i>	0.88 ± 0.26	0.85 ± 0.11	0.63 ± 0.16	0.33 ± 0.1*
<i>Pendrin</i>	0.83 ± 0.18	0.46 ± 0.08	0.74 ± 0.09	0.35 ± 0.05*

Values, normalized to β -actin, are expressed as mean $\pm$ S.E.M. of one representative experiment ($n=6$). * $P<0.05$ versus control cells.

likely in very low amounts, as suggested by previous models (Denef *et al.* 1996). Further studies are required to find out the source and the type of intracellular ROS, and to sort out those resulting from aerobic metabolism as in every other organs, and those specifically involved in hormone synthesis.

NAC may decrease cell OS, either directly, as a source of sulfhydryl groups that neutralize ROS, or indirectly as glutathione precursor by restoring glutathione levels (GSH) (Gillissen & Nowak 1998). The present study shows that NAC decreases intracellular ROS to very low levels without inducing apoptosis or affecting cell viability. This marked decrease in intracellular ROS levels is associated with decreased DUOXs, TPO, TG, and pendrin expressions, but not *Nis* mRNA expression. Such effects on DUOXs expression were also observed *in vivo*. As NAC may also influence GSH intracellular levels, it is possible that some specific biochemical processes might be altered by glutathionylation, i.e., the reversible formation of disulfide bonds

between protein cysteines and glutathione, as recently suggested (Cao *et al.* 2005, le-Donne *et al.* 2007). Although, ROS are sometimes known to act as transduction signals, for instance in cells stimulated by ligands (cytokines, growth factors), or in pathological situations (Lander 1997, Finkel 1998, Adler *et al.* 1999), it is the first time that the maintenance of a minimal intracellular oxidative load is shown to be required for safeguarding endocrine functions. This has already been suggested in pancreatic cells or in the lung (Lenzen 2008), but without any convincing demonstration. Thus, when the oxidative load in thyrocytes drops below a minimal threshold, various actors involved in the TH synthesis are also downregulated. This might eventually be relevant in terms of thyroid function in patients treated with NAC, but as far as we know, there is no robust data yet reporting such interactions in the literature.

In addition to ROS, thyrocytes also produce RNS in low amounts (basal nitrite release) associated with NOS2 and NOS3 expressions (Colin *et al.* 1995), suggesting a role for NO in normal thyroids. In contrast with ROS, NAC increased NO production without affecting *Nos2* mRNA expression. The interpretation of this result is somewhat difficult as NAC-induced effects on NOS2 expression remains a matter of debate. It may either increase, or decrease, or have no effect on NOS2 expression and NO release (Ramasamy *et al.* 1999, Vos *et al.* 1999, Chen *et al.* 2000, Zafarullah *et al.* 2003). Nevertheless, NO levels are particularly low when compared with those produced for instance in cells incubated with Th1 cytokines (Kasai *et al.* 1995, van den Hove *et al.* 2002, Gerard *et al.* 2006). Although this slight increase in NO production may appear odd in cells treated with NAC, one should keep in mind that superoxide anions, which are constantly produced

Figure 2 NAC differentially regulates the expression of antioxidant enzymes in human thyrocytes. PRDX5 (A), PRDX3 (C), and catalase (D) protein expressions were analyzed by western blot. Densitometric values normalized to β -actin are expressed as mean $\pm$ S.E.M. of one representative experiment ($n=6$). * $P<0.05$ versus control cells. PRDX5 protein (B) localization was analyzed by immunofluorescence in control- and NAC-treated cells. Scale bars: 20 μ m. Full colour version of this figure available via <http://dx.doi.org/10.1677/JOE-08-0470>.

Figure 3 NAC decreases DUOX and PRDX5 expressions in mouse thyroids. DUOXs protein expression was detected by immunohistochemistry on frozen sections. In control thyroids (A), DUOXs was detected at the apical pole of the cells (arrow). In NAC-treated mice (B), DUOXs was barely detected only in few cells. PRDX5 protein expression was detected by immunohistochemistry on paraffin sections. In control thyroids (C), PRDX5 was detected in the cytoplasm and in nuclei. In NAC-treated cells (D), the staining was greatly decreased. Scale bars, 10 µm. Full colour version of this figure available via <http://dx.doi.org/10.1677/JOE-08-0470>.

by mitochondria during aerobic respiration (Bedard & Krause 2007), react with NO in normal cells to form low amounts of nitrotyrosines. Although this remains to be proven, it could be speculated that in case of NAC-induced ROS suppression, superoxide anions are no more available in sufficient amounts to react with NO. Consequently, NO might then be released in culture media in slightly greater amounts.

As the oxidative load was deeply affected by NAC, it made sense to analyze how antioxidant systems react in such conditions. Our results indicate that they are actually influenced by intracellular ROS, especially PRDXs whose expression is known to be regulated by OS (Bast *et al.* 2002). In our study, PRDX5 expression decreased, while PRDX3 increased in NAC-treated cells, indicating that both proteins are differently regulated in conditions of ROS depletion. By contrast, catalase protein expression remained unchanged, which is in line with the low enzymatic activity previously reported in the thyroid (Nadolnik & Valentyukovich 2007). Thus, in accordance with previous results (Gerard *et al.* 2005, Poncin *et al.* 2008a), our present data confirm that PRDXs 5 and 3 are in fact highly regulated in the thyroid, suggesting that they may play important roles in detoxification processes along side the glutathione peroxidase system (Nadolnik & Valentyukovich 2007).

In conclusion, our results showed for the first time that in physiological conditions, thyroid cells produce intracytoplasmic ROS. When their intracellular levels drop very low, as in NAC-treated cells, the expression of important proteins involved in TH synthesis is hampered. This strongly suggests that the maintenance of the oxidative load above a minimum threshold is required to safeguard the function of thyroid cells.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References

- Adler V, Yin Z, Tew KD & Ronai Z 1999 Role of redox potential and reactive oxygen species in stress signaling. *Oncogene* **18** 6104–6111.
- Banmeyer I, Marchand C, Clippe A & Knoops B 2005 Human mitochondrial peroxiredoxin 5 protects from mitochondrial DNA damages induced by hydrogen peroxide. *FEBS Letter* **579** 2327–2333.
- Bast A, Wolf G, Oberbauer I & Walther R 2002 Oxidative and nitrosative stress induces peroxiredoxins in pancreatic beta cells. *Diabetologia* **45** 867–876.
- Bedard K & Krause KH 2007 The NOX, family of ROS-generating NADPH oxidases: physiology and pathophysiology. *Physiological Reviews* **87** 245–313.
- Bjorkman U & Ekholm R 1992 Hydrogen peroxide generation and its regulation in FRTL-5 and porcine thyroid cells. *Endocrinology* **130** 393–399.
- Blokhina O, Virolainen E & Fagerstedt KV 2003 Antioxidants, oxidative damage and oxygen deprivation stress: a review. *Annals of Botany* **91** 179–194.
- Cao X, Kambe F, Lu X, Kobayashi N, Ohmori S & Seo H 2005 Glutathionylation of two cysteine residues in paired domain regulates DNA binding activity of Pax-8. *Journal of Biological Chemistry* **280** 25901–25906.
- Chen G, Wang SH & Warner TD 2000 Regulation of iNOS mRNA levels in endothelial cells by glutathione, a double-edged sword. *Free Radical Research* **32** 223–234.
- Colin IM, Nava E, Toussaint D, Maiter DM, vanDenhove MF, Luscher TF, Ketelslegers JM, Denef JF & Jameson JL 1995 Expression of nitric oxide synthase isoforms in the thyroid gland: evidence for a role of nitric oxide in vascular control during goiter formation. *Endocrinology* **136** 5283–5290.
- Contempe B, Denef JF, Dumont JE & Many MC 1993 Selenium deficiency aggravates the necrotizing effects of a high iodide dose in iodine deficient rats. *Endocrinology* **132** 1866–1868.
- Contempe B, de Escobar GM, Denef JF, Dumont JE & Many MC 2004 Thiocyanate induces cell necrosis and fibrosis in selenium- and iodine-deficient rat thyroids: a potential experimental model for myxedematous endemic cretinism in central Africa. *Endocrinology* **145** 994–1002.
- Dai G, Levy O & Carrasco N 1996 Cloning and characterization of the thyroid iodide transporter. *Nature* **379** 458–460.
- De Deken X, Wang D, Dumont JE & Miot F 2002 Characterization of ThOX proteins as components of the thyroid H₂O₂-generating system. *Experimental Cell Research* **273** 187–196.
- Denef JF, Many MC & van den Hove MF 1996 Iodine-induced thyroid inhibition and cell necrosis: two consequences of the same free-radical mediated mechanism? *Molecular Cellular Endocrinology* **121** 101–103.
- Dupuy C, Ohayon R, Valent A, Noel-Hudson MS, Deme D & Virion A 1999 Purification of a novel flavoprotein involved in the thyroid NADPH oxidase. Cloning of the porcine and human cDNAs. *Journal of Biological Chemistry* **274** 37265–37269.

- le-Donne I, Rossi R, Giustarini D, Colombo R & Milzani A 2007 S-glutathionylation in protein redox regulation. *Free Radical Biology and Medicine* **43** 883–898.
- Finkel T 1998 Oxygen radicals and signaling. *Current Opinion in Cell Biology* **10** 248–253.
- Fortemaison N, Miot F, Dumont JE & Dremier S 2005 Regulation of H₂O₂ generation in thyroid cells does not involve Rac1 activation. *European Journal Endocrinology* **152** 127–133.
- Fusco A, Berlingieri MT, Di Fiore PP, Portella G, Grieco M & Vecchio G 1987 One- and two-step transformations of rat thyroid epithelial cells by retroviral oncogenes. *Molecular and Cellular Biology* **7** 3365–3370.
- Gerard AC, Many MC, Daumerie C, Knoops B & Colin IM 2005 Peroxiredoxin 5 expression in the human thyroid gland. *Thyroid* **15** 205–209.
- Gerard AC, Boucquoy M, van den Hove MF & Colin IM 2006 Expression of TPO and ThOXs in human thyrocytes is downregulated by IL-1 α /IFN- γ , an effect partially mediated by nitric oxide. *American Journal of Physiology-Endocrinology and Metabolism* **291** E242–E253.
- Gerard AC, Poncin S, Caetano B, Sonveaux P, Audinot JN, Feron O, Colin IM & Soncin F 2008 Iodine deficiency induces a thyroid stimulating hormone-independent early phase of microvascular reshaping in the thyroid. *American Journal of Pathology* **172** 748–760.
- Gillissen A & Nowak D 1998 Characterization of N-acetylcysteine and ambroxol in anti-oxidant therapy. *Respiratory Medicine* **92** 609–623.
- van den Hove MF, Stoenoiu MS, Croizet K, Couvreur M, Courtroy PJ, Devuyt O & Colin IM 2002 Nitric oxide is involved in interleukin-1 α -induced cytotoxicity in polarised human thyrocytes. *Journal of Endocrinology* **173** 177–185.
- Kasai K, Hattori Y, Nakanishi N, Manaka K, Banba N, Motohashi S & Shimoda S 1995 Regulation of inducible nitric oxide production by cytokines in human thyrocytes in culture. *Endocrinology* **136** 4261–4270.
- Krohn K, Maier J & Paschke R 2007 Mechanisms of disease: hydrogen peroxide, DNA damage and mutagenesis in the development of thyroid tumors. *Nature Clinical Practice. Endocrinology & Metabolism* **3** 713–720.
- Lander HM 1997 An essential role for free radicals and derived species in signal transduction. *FASEB Journal* **11** 118–124.
- Lenzen S 2008 Oxidative stress: the vulnerable beta-cell. *Biochemical Society Transactions* **36** 343–347.
- Maier J, van SH, van OC, Paschke R, Weiss RE & Krohn K 2007 Iodine deficiency activates antioxidant genes and causes DNA damage in the thyroid gland of rats and mice. *Biochimica et Biophysica Acta* **1773** 990–999.
- Mano T, Shinohara R, Iwase K, Kotake M, Hamada M, Uchimuro K, Hayakawa N, Hayashi R, Nakai A, Ishizuki Y *et al.* 1997 Changes in free radical scavengers and lipid peroxide in thyroid glands of various thyroid disorders. *Hormone and Metabolic Research* **29** 351–354.
- Mutaku JF, Poma JF, Many MC, Denef JF & van den Hove MF 2002 Cell necrosis and apoptosis are differentially regulated during goitre development and iodine-induced involution. *Journal of Endocrinology* **172** 375–386.
- Nadolnik LI & Valentyukevich OI 2007 Peculiarities of the antioxidant status of the thyroid gland. *Bulletin of Experimental Biology and Medicine* **144** 529–531.
- Poncin S, Gerard AC, Boucquoy M, Senou M, Calderon PB, Knoops B, Lengele B, Many MC & Colin IM 2008a Oxidative stress in the thyroid gland: from harmlessness to hazard depending on the iodine content. *Endocrinology* **149** 424–433.
- Poncin S, Lengele B, Colin IM & Gerard AC 2008b Differential interactions between Th1/Th2, Th1/Th3, and Th2/Th3 cytokines in the regulation of TPO and DUOX expression, and of thyroglobulin secretion in thyrocytes *in vitro*. *Endocrinology* **149** 1534–1542.
- Ramasamy S, Drummond GR, Ahn J, Storek M, Pohl J, Parthasarathy S & Harrison DG 1999 Modulation of expression of endothelial nitric oxide synthase by nordihydroguaiaretic acid, a phenolic antioxidant in cultured endothelial cells. *Molecular Pharmacology* **56** 116–123.
- Schomburg L & Kohrle J 2008 On the importance of selenium and iodine metabolism for thyroid hormone biosynthesis and human health. *Molecular Nutrition & Food Research* **52** 1235–1246.
- Schweizer U, Chiu J & Kohrle J 2008 Peroxides and peroxide-degrading enzymes in the thyroid. *Antioxidants & Redox Signaling* **10** 1577–1592.
- Vos TA, Van GH, Tuyt L, De Jager-Krikken A, Leuvenink R, Kuipers F, Jansen PL & Moshage H 1999 Expression of inducible nitric oxide synthase in endotoxemic rat hepatocytes is dependent on the cellular glutathione status. *Hepatology* **29** 421–426.
- Zafarullah M, Li WQ, Sylvester J & Ahmad M 2003 Molecular mechanisms of N-acetylcysteine actions. *Cellular and Molecular Life Sciences* **60** 6–20.

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