Iodide excess regulates its own efflux: a possible involvement of pendrin

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Am J Physiol Cell Physiol 310: C576–C582, 2016. First published January 20, 2016; doi:10.1152/ajpcell.00210.2015.—Adequate iodide supply and metabolism are essential for thyroid hormone synthesis. In thyrocytes, iodide uptake is mediated by the sodium-iodide symporter, but several proteins appear to be involved in iodide efflux. Previous studies demonstrated that pendrin is able to mediate apical efflux of iodide in thyrocytes. Acute iodide excess transiently impairs thyroid hormone synthesis, a phenomenon known as the Wolff-Chaikoff effect. Although the escape from this inhibitory effect is not completely understood, it has been related to the inhibition of sodium-iodide symporter-mediated iodide uptake. However, the effects of iodide excess on pendrin efflux have not been characterized. Herein, we investigated the consequences of iodide excess on pendrin abundance, subcellular localization, and iodide efflux in rat thyroid PCCl3 cells. Our results indicate that iodide excess increases pendrin abundance and plasma membrane insertion after 24 h of treatment. Moreover, iodide excess increases pendrin half-life. Finally, iodide exposure also increases iodide efflux from PCCl3 cells. In conclusion, these data suggest that pendrin may have an important role in mediating iodide efflux in thyrocytes, especially under conditions of iodide excess.

IODIDE IS ESSENTIAL FOR THE biosynthesis of thyroid hormones, which exert a major impact on development, growth, and metabolism (21, 47). Thyroid hormone synthesis requires adequate nutritional supply, transport, and metabolism of iodide in the thyrocytes. Iodide uptake across the basolateral membrane is mediated by the sodium-iodide symporter (NIS) (29), while the efflux across the apical membrane is mediated, at least in part, by pendrin, the protein encoded by the Pendred syndrome (PDS/SLC26A4) gene (2, 33).

Acute iodide excess impairs thyroid hormone synthesis, a phenomenon known as the Wolff-Chaikoff effect (25, 45). This effect is transitory and, even though the mechanisms that underlie the escape of the inhibitory effect are still not completely understood, it has been related to a decrease of NIS expression and activity (12, 13, 34, 35). Both the Wolff-Chaikoff effect and the escape from this transient inhibition are necessary to maintain thyroid hormone synthesis under tight control, and they are important for protecting the individual from iodine excess (4, 30). Consistent with an autoregulatory phenomenon, several studies have shown that iodide regulates the expression of genes that encode proteins involved in thyroid hormone synthesis (7, 23, 40).

Although much is known about the mechanisms involved in the regulation of iodide uptake in response to iodide excess (1, 24, 36), less is known about the effects of iodide exposure on its own efflux at the apical membrane. Studies performed with vesicles obtained from thyroid cells have suggested the presence of two distinct apical iodide channels or exchangers (18), but their molecular identity remains uncertain. Several studies suggest that pendrin is involved in mediating iodide efflux at the apical membrane into the follicular lumen (11, 15, 32, 37, 41, 48, 49). Supporting a role of pendrin in thyroidal iodide transport, biallelic mutations in the PDS/SLC26A4 gene cause the Pendred syndrome, an autosomal recessive disorder that is characterized by sensorineural hearing impairment, presence of goiter, and a partial defect in iodide organization (9, 10, 27). However, the absence of thyroid abnormalities in some Pendred syndrome patients suggests that at least one other channel/exchanger is involved in the apical transport of iodide. A second channel that may be involved in apical iodide efflux is anotamin 1 (ANO1) (19, 39). Recent studies have demonstrated that ANO1 is expressed at the apical membrane of human and rat thyroid cells, and functional studies demonstrated its ability to mediate iodide efflux in PCCl3 and FRTL-5 rat thyroid cells, as well as in transfected heterologous cell lines (19, 39). The function of ANO1 is stimulated by the diacylglycerol-calcium pathway, whereas the membrane insertion of pendrin is stimulated by thyroid stimulating hormone (28). Our laboratory has previously shown an increase of Pds/Slc26a4 mRNA expression under conditions of iodide excess (7), a finding suggesting that this multifunctional anion exchanger could play an important role in this specific circumstance.

Taking into account this previous observation, we investigated the consequences of acute iodide excess on pendrin content, subcellular localization, and autoregulation of its own efflux in rat thyroid PCCl3 cells.

MATERIALS AND METHODS

Cell culture and treatments. Rat thyroid PCCl3 cells were cultured in Coon’s modified Ham’s F-12 medium supplemented with 5% fetal bovine serum, thyroid stimulating hormone (1 μU/ml), insulin (10 μg/ml), apotransferrin (5 μg/ml), and hydrocortisone (10 nM) (Sigma Aldrich, St. Louis, MO). When cells achieved 80% of confluence, the medium was replaced by fresh medium supplemented or not with sodium iodide (NaI; 10⁻³ to 10⁻⁹ M). The cells were maintained at this experimental condition for 30 min, or 1, 24, or 48 h. To evaluate...
the half-life of pendrin, PCCl3 cells were incubated for 1 h with 10 µg/ml cycloheximide (42) and then treated or not with 10⁻³ M NaI for 0, 3, 6, 8, or 10 h. Cell lysates were prepared, and the expression of pendrin was analyzed by Western blotting, as described below.

**Protein extraction and Western blot analysis.** PCCl3 cells were scraped in RIPA buffer (20 mM Tris, pH 7.5; 150 mM NaCl; 1% Nonidet P-40; 0.5% sodium deoxycholate; 1 mM EDTA, and 0.1% SDS) and a protease inhibitor cocktail (28). Proteins were quantified by Bradford assay (Bio-Rad, Hercules, CA). Subsequently, 40 µg of total protein were resolved by 8% SDS-PAGE, transferred onto polyvinylidene difluoride membranes that were blocked in 5% nonfat milk, and incubated with a specific polyclonal chicken IgY anti-pendrin antibody (17, 28). A horseradish peroxidase-conjugated secondary goat anti-chicken IgY (Aves Labs, Tigard, OR) was used as secondary antibody. Total pendrin expression was analyzed by using the Bio-Rad enhanced chemiluminescence detection kit (Bio-Rad, Hercules, CA). Blot densitometry was performed by using the ImageJ Software (National Institutes of Health, Bethesda, MD).

**Immunofluorescence analysis.** PCCl3 cells were seeded on cover glass slides in poly-D-lysine cell-ware six-well plates (5 × 10⁵ cells/well). After the treatment with 10⁻³ M NaI for 2, 12, 24, and 48 h, the cells were washed three times with ice-cold PBS/CM (PBS supplemented with 0.1 mM CaCl₂ and 1 mM MgCl₂). The cells were fixed at room temperature with 2% paraformaldehyde diluted in PBS for 20 min. Thereafter, the cells were incubated for 2 h under nonpermeabilized conditions with an antibody directed against the extracellular epitopes of pendrin (1:200; Genovac, Freiburg, Germany) (28). Subsequently, cells were washed twice with PBS/CM and incubated for 1 h with an anti-rabbit IgG-FITC antibody (Sigma Aldrich, St. Louis, MO). The fluorescence was analyzed with a ZEISS Axiovert 100M fluorescence microscope.

To validate the nonpermeabilized condition, fixed PCCl3 cells were incubated with an anti-thyroid transcription factor 1 (TTF-1) antibody (1:200; Santa Cruz Biotechnology, Santa Cruz, CA), which recognizes the nuclear TTF-1 (TTF-1/NKX2.1). Both nonpermeabilized and permeabilized conditions were tested. The permeabilization was performed by incubating the fixed PCCl3 cells with 0.2% Triton diluted in PBS for 15 min.

**Flow cytometry analysis.** After the specified treatments, PCCl3 cells were fixed in 2% paraformaldehyde for 20 min and washed twice.

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**Fig. 1.** Pendrin protein abundance in PCCl3 rat thyroid cells is increased by treatment with 10⁻³ M sodium iodide (NaI; A), but not by 10⁻⁶ M (B), 10⁻⁷ M (C), or 10⁻⁹ M (D). Western blot analysis of total pendrin content was performed in cells treated or not [control (C)] for 30 min, or 1, 24, or 48 h with 10⁻³, 10⁻⁶, 10⁻⁷, or 10⁻⁹ M NaI. Pendrin levels were normalized to β-actin content. Immunoblots shown are representative of at least 3 independent experiments. Values are means ± SE in arbitrary units (AU). ****p < 0.0001 vs. C (ANOVA, Student-Newman-Keuls).
with PBS. Subsequently, the cells were incubated for 1 h, under nonpermeabilized conditions, with an antibody directed against the extracellular epitopes of pendrin (1:500; Genovac, Freiburg, Germany). The cells were washed twice with PBS and incubated with an anti-rabbit IgG-FITC antibody (1:100; Sigma Aldrich, St. Louis, MO). The fluorescence of $10^4$ events per sample was acquired using a Guava easyCyte flow cytometer and analyzed with GuavaExpress Pro Software (EMD Millipore, Billerica, MA).

**Iodide efflux assay.** To evaluate iodide efflux in cells exposed to NaI, we performed two sets of experiments. First, PCCl3 cells were incubated with $10^{-3}$ M NaI for 1, 4, and 6 h. Second, the cells were exposed to iodide excess for longer periods of treatment (12, 24, and 48 h).

In the experiments with short periods of iodide exposure, the cells were incubated for 30 min with 500 μl of prewarmed (37°C) serum-free culture medium containing $10^{-3}$ M nonradioactive NaI and 20 μCi/μmol I⁻ carrier-free Na125I after the specified treatment period. To determine the iodide efflux in the experiments conducted with longer periods of iodide excess, PCCl3 cells were incubated with $10^{-3}$ M nonradioactive NaI and 20 μCi/μmol I⁻ carrier-free Na125I for 12, 24, and 48 h. This allowed the accumulation of radioactive iodide inside the cells before the exposure to the different treatments. The control cells were incubated with medium containing $10^{-3}$ M nonradioactive NaI and 20 μCi/μmol I⁻ carrier-free Na125I for 30 min. After the periods of exposure described above, the cells were quickly washed with warm PBS. Subsequently, medium containing 1 mM sodium perchlorate was added to suppress iodide re-uptake. The cells were incubated with the perchlorate solution for 90 s (T1). After that, the solution was removed, and radioactivity was quantified in a gamma-counter. This step was repeated twice (T2 and T3). Finally, the PCCl3 cells were lysed using a 1% Triton diluted in PBS.

$^{125}$I activities of each fraction (T1, T2, T3, and cell lysate) were summed and considered as the total amount of iodide (100%) that was transported into the thyrocytes, as described (43). With this information, it was possible to calculate the rate of iodide efflux at T1, T2, and T3 of the control and iodide-treated cells, independently of the fact that NIS was inhibited in cells pretreated with iodide. For standardization, the DNA amount of each well was determined (16).

**RNA extraction and real-time PCR analysis.** Total RNA of PCCl3 cells was purified using Trizol. Real-time PCR amplifications were performed using Platinum SYBR Green qPCR Super Mix-UDG (Life Technologies, Carlsbad, CA). Relative changes in Ano1 mRNA expression in iodide-treated cells were calculated using the $2^{-\Delta \Delta Ct}$ method and Rpl19 as internal control.

**Statistical analysis.** All data are reported as means ± SE. The significance level was set at 5% ($P < 0.05$). The data were subjected to unpaired two-tailed Student’s t-test or one-way ANOVA, followed by Student-Newman-Keuls posttest (GraphPad Prism Software, San Diego, CA, version 6.0), when appropriate.

**RESULTS**

**Effect of iodide excess treatment on pendrin expression.** The exposure of PCCl3 cells to $10^{-3}$ M NaI for 24 h significantly increased pendrin expression compared with the control group (Fig. 1A). This effect was not observed in cells treated with lower doses of iodide ($10^{-5}$ M, $10^{-7}$ M, and $10^{-9}$ M NaI), as shown in Fig. 1, B–D.

**Fig. 2.** Treatment with iodide excess increases pendrin insertion at the membrane of PCCl3 cells. Thyroid cells were incubated or not (C) with $10^{-3}$ M NaI for 2, 12, 24, or 48 h. Cells were fixed and incubated with an antibody directed against the extracellular epitopes of pendrin under nonpermeabilized condition. Thereafter, the cells were incubated with an anti-rabbit IgG-FITC antibody. Pictures are representative of 2 independent experiments. Immunofluorescence was analyzed with a ZEISS Axiovert 100M fluorescence microscope. Green signal, pendrin; blue signal, 4’,6-diamidino-2-phenylindole. Magnification ×20.
Effect of iodide excess exposure on subcellular localization of pendrin. The localization of pendrin was analyzed in cells treated with $10^{-3}$ M NaI for 2, 12, 24, or 48 h. The immunofluorescence analysis, performed under nonpermeabilized conditions, revealed stronger fluorescence intensity in cells treated with iodide excess for 12, 24, and 48 h (Fig. 2). The integrity of a nonpermeabilized condition was confirmed by the absence of TTF-1 labeling in the nucleus of thyroid cells (data not shown).

To confirm the immunofluorescence data, we performed flow cytometry analysis under nonpermeabilized conditions with PCCl3 cells. The results demonstrate that iodide-treated thyroid cells display increased amounts of pendrin at the plasma membrane after 24 and 48 h of exposure (Fig. 3).

Effect of iodide excess treatment on pendrin half-life. Since we have observed increased amounts of pendrin in response to iodide excess, we investigated whether this treatment could influence pendrin turnover. After 10 h in the presence of cycloheximide, the remaining amount of pendrin in iodide-treated cells was $\sim80\%$, whereas in the control cells the remaining amount was $\sim50\%$ (Fig. 4). These results suggest that iodide excess increases pendrin half-life. Pendrin content was normalized to the corresponding total protein amount determined by densitometric analysis after Ponceau staining (data not shown).

Effect of iodide excess on iodide efflux. Consistent with the data on pendrin abundance in the plasma membrane, treatment with high amounts of iodide for short periods of time did not alter the efflux of iodide compared with the control group (Fig. 5A). In contrast, the rate of iodide efflux was higher in cells treated with iodide for longer time periods (12–48 h) (Fig. 5B). In both instances, the iodide efflux was more prominent at 90 s, when the first fraction was collected (T1).

It is worth noting that the iodide uptake was substantially decreased after iodide excess treatment for 12, 24, and 48 h (data not shown), and, despite the low amount of intracellular iodide, the efflux was higher in these groups.

Effect of iodide excess treatment on ANO1 mRNA expression. The exposure of PCCl3 cells to $10^{-3}$ M NaI for 24 h did not alter Ano1 mRNA expression (Fig. 6). In contrast, in a previous study, we have shown a significant increase in the expression of Pds/Slc26a4 mRNA in response to iodide exposure (7).

DISCUSSION

It is well known that iodide excess inhibits NIS expression and activity, resulting in a reduction of NIS-mediated iodide uptake (1, 8, 12, 13, 36). Although several studies have been performed to investigate the molecular mechanisms involved in regulating iodide uptake by thyrocytes, the effects of iodide excess on its own efflux have drawn less attention and are poorly elucidated. Here, we show that iodide excess increases the total and plasma membrane content of pendrin, as well as iodide efflux in PCCl3 cells. This suggests that pendrin might play an important role in reducing intracellular iodide content...
when the supply of this trace element is excessive, preventing potentially deleterious effects on thyroid cells.

Two apical iodide channels are thought to be involved in iodide efflux in thyroid cells based on studies with membrane vesicles (18). Electrophysiological studies suggested that one of these channels has a high permeability and specificity for iodide \(
\frac{K_m}{H_i} \approx 70 \text{ mM}
\), while the second has a much lower affinity for this anion \( \frac{K_m}{H_i} \approx 33 \text{ mM} \). The molecular identity of the two channels remains, however, uncertain. Pendrin may represent one of these channels/exchangers. However, the exact physiological role of pendrin in mediating iodide efflux is still controversial. Several studies suggest a role of pendrin in mediating iodide efflux into the follicular lumen (17, 22, 38). Moreover, the thyrocytes of patients with Pendred syndrome display low amounts of iodide in the follicular lumen (27). In contrast to the human condition, \( Pds \) knockout mice do not develop goiters or hypothyroidism, even under conditions of low iodide intake (6, 14, 20).

Studies performed in our laboratory have shown that iodide excess increases \( Pds/Slc26a4 \) mRNA content after treatment with iodide for 30 min to 48 h in \( PCCl_3 \) cells (7). Two recent studies have shown that a \( \text{Ca}^{2+} \)-activated anion channel, \( \text{ANO1} \), mediates iodide efflux across the apical membrane of thyroid cells under basal conditions (19, 39). It is possible, although not formally proven, that \( \text{ANO1} \) and pendrin could reflect the two apical iodide transporters. Currently, their relative affinity for iodide is unknown.

In the present study, we analyzed whether pendrin abundance and function are regulated by high-iodide concentrations in \( PCCl_3 \) cells. High-iodide concentrations lead to increased pendrin abundance after 24 h of treatment and only with the highest NaI concentration used \( (10^{-3} \text{ M}) \) (Fig. 1). This change could be a consequence of increased pendrin synthesis, since

![Fig. 5. Iodide efflux in \( PCCl_3 \) cells treated for short periods (A) or longer periods (B) of iodide excess exposure. A: cells were incubated for 1, 4, and 6 h with \( 10^{-3} \text{ M} \) non-radioactive NaI. After that, 20 Ci/\( \mu \)mol \( \text{I}^- \) carrier-free Na\(^{125}\)I was added to the medium for 30 min. The medium was then replaced by medium containing perchlorate after 90, 150, and 210 s. B: cells were incubated with \( 10^{-3} \text{ M} \) nonradioactive NaI and 20 Ci/\( \mu \)mol \( \text{I}^- \) carrier-free Na\(^{125}\)I for 12, 24, or 48 h. After that, the medium was replaced by medium containing perchlorate after 90, 150, and 210 s. The radioactivity in each fraction was measured using a gamma-counter. The amount of \( ^{125}\text{I} \) was normalized by the amount of DNA in each sample. Values are means ± SE. Three independent experiments were performed in triplicate. **** \( p < 0.0001 \) vs. control (one-way ANOVA, Student-Newman-Keuls).](http://ajpcell.physiology.org/)

![Fig. 6. Iodide excess does not alter \( \text{Ano1} \) (anoctamin 1) mRNA expression. Relative \( \text{Ano1} \) mRNA expression was evaluated by real-time PCR. \( PCCl_3 \) cells were incubated with NaI \( (10^{-3} \text{ M}) \) for 24 h. Results are indicated as fold change relative to the mRNA levels of untreated cells. Values are means ± SE. \( P > 0.05 \) vs. control cells (unpaired two-tailed Student’s t-test).](http://ajpcell.physiology.org/)

![Fig. 7. Hypothetical mechanism involved in the escape of the Wolff-Chaikoff effect. In response to high intracellular iodide concentrations, sodium-iodide symporter (NIS) synthesis and activity is inhibited, while pendrin (Pendred syndrome [PDS]) is inserted more abundantly in the apical membrane, and its mRNA expression is stimulated, which results in increased iodide efflux under conditions of a high intracellular iodide concentration.](http://ajpcell.physiology.org/)
we have observed that iodide excess treatment augmented Pds/Slc26a4 mRNA expression, both in vivo and in vitro in a previous study (7). Moreover, as shown here, the half-life of pendrin was increased by exposure to iodide excess, which suggests that the degradation of pendrin is decreased under this treatment condition (Fig. 4). Hence, the change in half-life could also contribute to the observed increase of pendrin abundance. We did not observe a dose-response effect on pendrin expression in response to iodide, suggesting that the increase of its expression requires a high intracellular concentration of iodide. Similarly, the Wolff-Chaikoff effect depends on a high intracellular concentration of iodide (31). Previous studies revealed that a concentration of iodide of 10^{-3} M is required to activate the Wolff-Chaikoff effect (4, 5).

Additionally, as indicated by our immunofluorescence and flow cytometry studies, iodide excess for 24 h increased pendrin abundance at the plasma membrane of thyroid cells (Fig. 2). This finding suggests not only that the excess of iodide does increase the total amount of pendrin in PCC13 cells, but that it is also enhancing its insertion in the plasma membrane, which is essential for mediating iodide efflux (10). The increased amount of pendrin at the plasma membrane observed after 12, 24, and 48 h of iodide excess treatment explains the increased efflux observed in the functional studies.

The lack of a change in the total amount of pendrin at 48 h contrasts with the upregulation of its mRNA and will need further characterization in the future. While the reasons are currently unclear, it is, e.g., possible that the inhibition of NIS synthesis and activity under these experimental conditions leads to a decrease in intracellular iodide content, and that the stimulatory effect is, therefore, only short-lived.

The augmented expression and membrane insertion of pendrin and the increased iodide efflux correlate with the period of the Wolff-Chaikoff effect escape phenomenon (3, 46). In contrast, both pendrin plasma membrane insertion and iodide efflux were not altered after short periods of iodide exposure. Taken together, our data suggest that pendrin exerts a key role in mediating iodide efflux into the follicular lumen under conditions of iodide excess.

Figure 7 presents the hypothetic mechanisms that underlie the escape from the Wolff-Chaikoff effect, in which pendrin seems to be directly involved. During the Wolff-Chaikoff effect, thyroid hormone synthesis is blocked, and intracellular iodide is high. Because of this, NIS is inhibited, and iodide uptake is decreased. This is the main known mechanism triggering the escape phenomenon. We, therefore, suggest that pendrin could perhaps play a role in the escape phenomenon (Fig. 7). When in excess, intracellular iodide could also increase pendrin expression, intensifying iodide efflux by this, and perhaps other, proteins (Fig. 7). If correct, this could contribute to the decrease in the intracellular iodide concentration and the subsequent escape from the inhibitory effect mediated by iodide.

Although several studies have shown that pendrin has the ability to mediate iodide efflux (17, 37), other transporters have been suggested to be involved in this process (15, 44). It has been shown that Clec5−/− mice have a phenotype reminiscent of Pendred syndrome, and that the iodide efflux is impaired in thyrocytes (41), and more recently ANO1 has been identified as an apical iodide transporter (19, 39). Our results indicate that the treatment of PCC13 cells with 10^{-3} M NaI for 24 h had no effect on Ano1 mRNA expression (Fig. 6), while the expression of Pds/Slc26a4 mRNA was increased, as previously demonstrated (7). Therefore, it is tempting to speculate that the specific expression and function of these thyroidal iodide transporters/exchangers could differentially modulate iodide efflux under different conditions. Although PCC13 cells are not polarized, which remains a limitation of this and numerous other studies using this model system, they have been widely used in studies aiming to investigate the regulation of proteins in thyroid (23, 26, 28, 39). Despite this, the importance of cell polarization to understand physiological mechanisms on thyroid epithelial cells must be considered.

In conclusion, the data presented here suggest that iodide excess not only inhibits NIS-mediated iodide uptake, but also results in an increase of its efflux in thyroid cells through pendrin, which may be an effective iodide transporter under conditions of iodide excess.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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REGULATION OF PENDRIN BY IODIDE