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Pendrin Is an Iodide-Specific Apical Porter Responsible for Iodide Efflux from Thyroid Cells

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The Pendred syndrome gene encodes a 780-amino acid putative transmembrane protein (pendrin) that is expressed in the apical membrane of thyroid follicular cells. Although pendrin was shown to transport iodide and chloride using *Xenopus laevis* oocytes and Sf9 insect cells, there is no report using mammalian cells to study its role in thyroid function. We show here, using COS-7 cells and Chinese hamster ovary cells transfected with expression vectors encoding sodium iodide symporter or human Pendred syndrome gene cDNA and by comparison with studies using rat thyroid FRTL-5 cells, that pendrin is an iodide-specific transporter in mammalian cells and is responsible for iodide efflux in the thyroid. (*J Clin Endocrinol Metab* 87: 3356–3361, 2002)

PENDRED SYNDROME IS a genetic disorder associated with profound sensorineural hearing loss and thyroid goiter (1–4). It is one of the most common forms of syndromic deafness; affected individuals typically have structural anomalies of the inner ear (5, 6). Pendred syndrome is also characterized by an organification defect of iodide in the thyroid, accompanied by goiters and a positive perchlorate discharge test (3, 4).

In 1997, the Pendred syndrome gene (PDS) was identified by a positional cloning strategy (7). The approximately 5-kb PDS transcript showed striking tissue-specific expression, being highly expressed in the thyroid, kidney, and inner ear (7, 8). The gene consists of 21 coding exons and encodes a putative 780-amino acid protein (pendrin) with 11 or 12 transmembrane domains (7, 9). Pendrin has high homology to known sulfate transporters (7); however, by using 2 different expression systems, *Xenopus laevis* oocytes and Sf9 insect cells, it was demonstrated that pendrin transports iodide and chloride in a competitive manner (10) and that it mediates bicarbonate secretion, chloride/formate, and chloride/OH^-/HCO_3^- exchange that may be important in the kidney (11–13).

The function of pendrin in thyroid cells has not been determined. In mammalian cells, competitive transportation of iodide and chloride, as shown in other cell systems (10), may not be plausible, as chloride is one of the most abundant ions in serum and also in cytoplasm. In addition, the characteristics of ion channels and transporters are known to be modified when expressed in *X. laevis* oocytes (14, 15). Thus, we showed previously that physiological characteristics of the sodium/iodide symporter (NIS) expressed in COS-7 cells or Chinese hamster ovary (CHO) cells were quite similar to those in thyroid cells, whereas characteristics of NIS expressed in *X. laevis* oocytes were not (16).

As Pendred syndrome is characterized by a defect of iodide organification, it is presumed that pendrin is important for iodide transport at the thyroid cell membrane (7, 9). It is known that iodide uptake into thyroid cells is mediated by NIS (17), which is expressed on the basolateral membrane of follicular cells (18, 19). Previous study showed that pendrin is localized on the apical membrane of thyroid follicular cells (9, 18–20). Therefore, it is highly likely that pendrin is the apical transporter of iodide responsible for the iodide efflux from thyrocytes into the follicular lumen. In the present report we use mammalian cell systems to study the physiological function of human pendrin in ion transport and show that pendrin is an iodide-specific transporter in the thyroid that is responsible for iodide efflux.

Materials and Methods

Cells

Rat thyroid FRTL-5 cells were grown as previously reported (20–22). COS-7 cells were maintained in DMEM containing 10% fetal bovine serum, 2 mmol/liter L-glutamine, and 1% penicillin and streptomycin. Cell cultures were kept at 37 C in a 5% CO_2 incubator. COS-7 cells transiently transfected with an expression vector containing NIS cDNA (16, 23) or PDS cDNA (9) were named N-COS and P-COS, respectively. CHO cells stably expressing NIS (CHO-4J cells) were described previously (16, 23). CHO-4J cells were grown in 24-well cell culture plates (Corning, Inc., Corning, NY) at 37 C in a 5% CO_2 incubator in Ham's F-12 medium supplemented with 5% fetal calf serum. Cells were transfected with an expression vector containing PDS cDNA using Lipofectamine reagent when they reached approximately 70% density and were named P-4J.

Abbreviations: CHO, Chinese hamster ovary; HBSS, Hanks' balanced salt solution; N-COS, COS-7 cells expressing sodium iodide symporter; NIS, sodium iodide symporter; P-COS, COS-7 cells expressing pendrin; PDS, Pendred syndrome gene; TG, thyroglobulin.

Iodide uptake and efflux studies

Iodide uptake was measured as previously described (23). Briefly, cells grown in 24-well plates were incubated with 500 μl chloride-free buffer or Hanks' balanced salt solution (HBSS) incubation buffer with 0.01–10 mM radioactive NaI (specific activities, 2–20 mCi/mmol) at 37 C for 2 or 120 min. Chloride-free buffer consisted of 140 mM sodium gluconate, 5.4 mM potassium gluconate, 1.8 mM $CaOH_2$, 0.5% BSA, and 10 mM HEPES-NaOH (pH 7.4), and HBSS incubation buffer consisted of HBSS with 0.5% BSA and 10 mM HEPES-NaOH (pH 7.4). After incubation, cells were washed twice on ice with ice-cold buffer, then solubilized with 1 ml 0.1 M NaOH, 0.1% (wt/vol) sodium dodecyl sulfate, and 2% Na_2CO_3, and radioactivity was counted. Iodide efflux studies were performed according to the method described by Weiss *et al.* (24). Briefly, after incubating the cells with 10 μM NaI and 0.1 μCi $Na^{125}I$ in 500 μl HBSS incubation buffer at 37 C for 60 min and washing them once with 2 ml chloride-free or HBSS buffer, 500 μl fresh nonradioactive (chloride free or HBSS buffer) medium with or without 10 μM NaI were added, and the cells were kept at 37 C for 5 min. The medium and cells were collected, and radioactivity was counted separately. The DNA content of cells in each well was measured by a previously described method (25) and used for normalization of the iodide uptake.

Voltage-clamp studies

Single cells were voltage-clamped using the whole cell configuration of the patch-clamp technique as previously described (16, 26). Briefly, monolayer cells were perfused with Tyrode solution consisting of 140 mM NaCl, 5.4 mM KCl, 1.8 mM $CaC1_2$, 0.5 mM $MgCl_2$, 5 mM glucose, and 5 mM HEPES (pH 7.4) with or without 100 μM NaI. The glass suction pipette was 2 μm in diameter at the tip, and the resistance was 3–5 MΩ when filled with the internal solution. The series resistance was less than 8 MΩ, as examined from the time course of the capacitative current recorded at the start of the whole cell voltage clamp. The current and voltage signals were stored on a digital magnetic tape and analyzed. The liquid junction (−10 mV) was not corrected for all membrane potential recordings. The pipette was filled with an internal solution consisting of 90 mM CsOH, 100 mM aspartate, 1.0 mM NaH_2P0_4, 5.0 mM $MgC1_2$, 20 mM tetraethylammonium chloride, 10 mM EGTA, 10 mM ATP magnesium salt, and 10 mM HEPES (pH 7.4) with or without 200 μM or 2 mM NaI. The holding potential was −40 mV.

Statistical analysis

Values are the mean ± SD of these experiments where noted. Significance between experimental values was determined by *t* test or Mann-Whitney's *U* test, and differences were considered significant at $P < 0.05$.

Results

Iodide accumulation mediated by NIS and pendrin

Our previous studies using mammalian cells showed that iodide uptake at 2 min represents the initial velocity, and uptake at 2 h represents stable equilibrium of iodide influx and efflux (23). Therefore, we first compared the ability of NIS and pendrin to cause accumulation of iodide at 2 min and 2 h after exposure to iodide. Extracellular iodide concentrations between 10 μM to 10 mM, which covers the range that NIS and iodide channels transport iodide (16, 27–29), were tested.

When iodide uptake was measured at 2 min after exposure to iodide, N-COS showed significantly higher uptake of iodide at all concentrations tested compared with the control cells ($P < 0.05$; Fig. 1A). Iodide uptake in N-COS cells increased in a dose-dependent manner up to 10 mM (Fig. 1A). On the other hand, P-COS showed no significant uptake at concentrations lower than 100 μM. However, uptake increased significantly at high iodide concentrations, and interestingly, it became higher than that of N-COS cells ($P < 0.05$ at 1 and 10 mM, at 2 min; Fig. 1A). The latter results were quite different from those of a previous report using *X. laevis* oocytes expressing pendrin, in which iodide transport was detected at 30 μM (10). High iodide uptake observed in control cells at high iodide concentrations is probably due to transport of iodide through an iodide-permeable chloride channel.

At equilibrium 2 h after exposure to iodide, N-COS cells showed higher iodide uptake than at 2 min for low concentrations, but uptake was similar to that at 2 min for higher concentrations (10 mM), suggesting that uptake is saturated at higher concentrations even 2 min after exposure to iodide. Unlike N-COS cells, iodide uptake in P-COS cells at low concentrations did not increase at 2 h compared with that at 2 min and showed similar uptake at high concentrations (Fig. 1B). When measured 2 h after exposure to iodide, iodide uptake in P-COS cells was not higher than that in N-COS cells in all iodide concentrations. These results indicate that efflux of iodide is higher in P-COS cells. If pendrin mediates iodide efflux at a high intracellular concentration of iodide, one might expect a decrease in intracellular iodide in P-COS cells

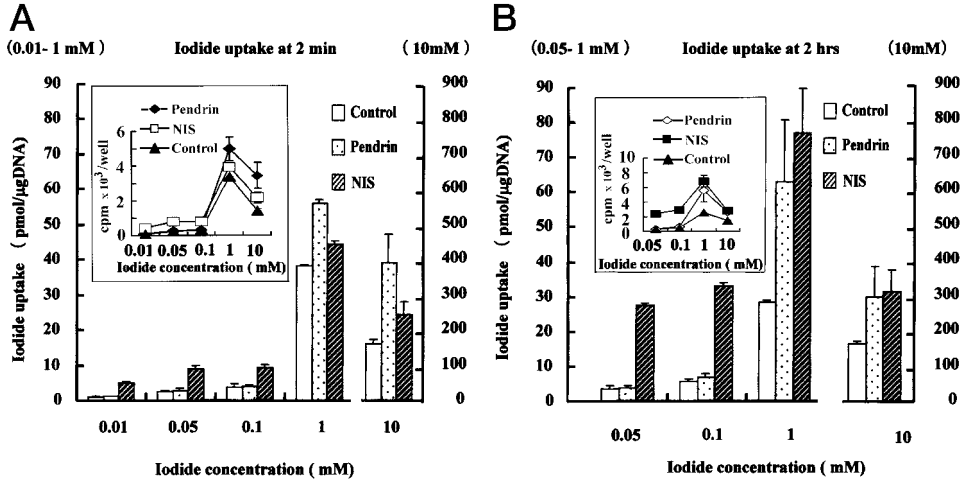

FIG. 1. $^{125}I^-$ uptake of P-COS and N-COS cells. COS-7 cells grown in 24-well plates transfected with expression vectors containing PDS cDNA (P-COS cells), NIS cDNA (N-COS cells), or vector alone (control) were incubated with 10 μM to 10 mM $Na^{125}I$ in chloride free buffer for 2 min (A) or 2 h (B). After incubation, cells were washed and solubilized to count radioactivity. DNA content was measured in a separate well and used to normalize the radioactivity. Results are expressed as the mean ± SD of five wells. In the inlets, the *ordinate* indicates net counts per minute of each well. Specific activity of $Na^{125}I$ was 20 mCi/mmol for the iodide concentration between 0.01–1 mM and 2 mCi/mmol for 10 mM, respectively.

compared with that in N-COS in the experiment shown in Fig. 1. The reason why a decrease in intracellular iodide in P-COS compared with that in N-COS cells was not observed would be the higher iodide influx through pendrin compared with NIS and a partial disturbance of iodide efflux under experimental conditions where a high extracellular iodide concentration exists in the medium.

Effect of chloride on $^{125}I^-$ uptake by pendrin

To confirm the results obtained from COS-7 cells transfected with NIS or PDS cDNAs, we performed similar studies using rat thyroid FRTL-5 cells that express endogenous NIS and pendrin and can transport iodide, because it is possible that pendrin changes its character when expressed on the cell membrane of cells from different organs, and it is also possible that the change in ion distribution induced by sodium and iodide transport by NIS can modify the effect of chloride.

Iodide uptake of FRTL-5 cells was measured in the presence or absence of 140 mM chloride, as a published study using *X. laevis* oocytes indicated that pendrin transports both iodide and chloride and that chloride displaces the uptake of iodide at a concentration of 30 μM (10). FRTL-5 cells showed a higher iodide uptake compared with N-COS and P-COS cells, especially at low concentrations (Fig. 2, A and B).

Iodide uptake in FRTL-5 cells was higher in the presence of 140 mM chloride at iodide concentrations between 0.01–1 mM (Fig. 2, A and B), suggesting that iodide and chloride are not competitively transported in FRTL-5 cells. The absence of chloride in culture medium will increase membrane potential by an extracellular shift of chloride ion. An increase in membrane potential will decrease iodide uptake by NIS, because iodide transport by NIS is membrane potential dependent (23).

To confirm that iodide and chloride are not competitively transported by pendrin, we tested the effect of chloride on P-COS cells incubated with various concentrations of chloride for 2 h in the presence of 1 mM iodide. There was no effect of chloride on iodide uptake by pendrin at any concentration tested (Fig. 3), indicating that there is no competitive transport of chloride and iodide by pendrin when expressed in mammalian cells.

Iodide efflux by pendrin

Our previous studies demonstrated that FRTL-5 cells release 77% of iodide within 3 min ($t_{1/2}$ = 2 min), whereas CHO cells stably expressing NIS (4J cells) release only 13% ($t_{1/2}$ = 15 min) (23). The low release of iodide in 4J cells indicates the lack of a mechanism for iodide efflux. To examine the effect of pendrin on iodide efflux, we compared iodide efflux of 4J cells and 4J cells transfected with PDS cDNA (P-4J cells) at 5 min in the presence or absence of 10 μM iodide or 140 mM chloride. Although there was some spontaneous iodide release in both cells, significantly higher iodide efflux was observed ($P < 0.01$) in P-4J cells over 4J cells in the presence of iodide (Fig. 4). This indicated that iodide efflux requires continuous inward flow of iodide to keep a high concentration of iodide in the cytoplasm. There was no effect of chloride on iodide efflux (Fig. 4), which is consistent with the results using FRTL-5 cells and COS-7 cells. These results strongly suggest that pendrin is responsible for iodide efflux, and that such a function depends on iodide, but not chloride, in mammalian cells.

The results described above demonstrate that pendrin transports iodide only at high concentrations. One of the questions, therefore, is whether NIS can accumulate iodide in high enough concentrations to initiate iodide transport of pendrin. To elucidate this point, we performed whole cell voltage-clamp studies using 4J cells under conditions of intracellular iodide at 200 μM and 2 mM. As shown in Fig. 5, the holding current at −40 mV rapidly shifted inwardly after switching the bathing solution to Tyrode solution with 100 μM NaI at both intracellular iodide concentrations. Such a response disappeared after the removal of NaI, and the results were the same as when there was no intracellular iodide. These results suggest that thyroid cells may be capable

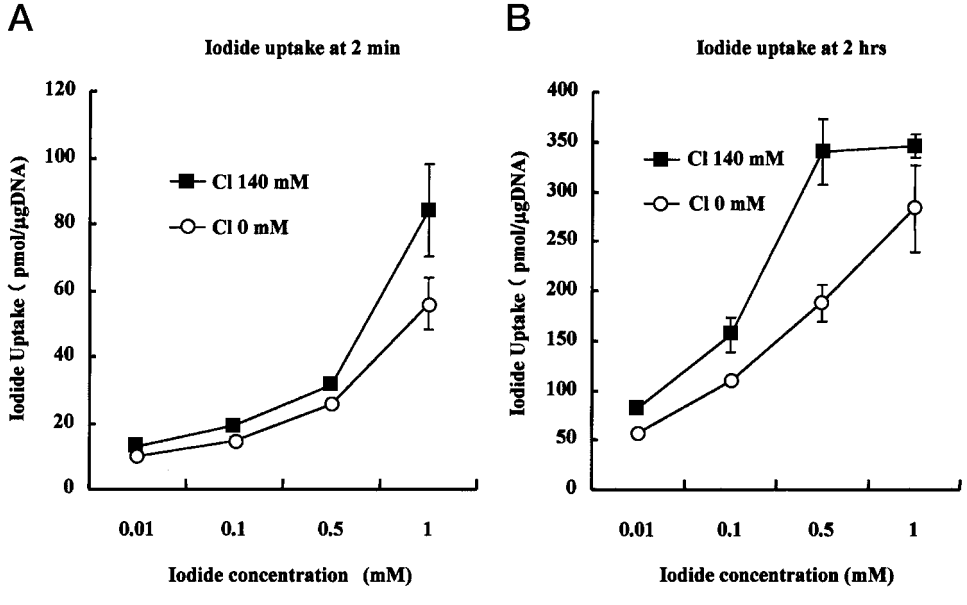

FIG. 2. Effect of chloride on $^{125}I^-$ uptake in FRTL-5 cells. FRTL-5 cells were incubated with $Na^{125}I$ for 2 min (A) or 2 h (B) in the presence or absence of chloride. After incubation, cells were washed and solubilized to count radioactivity. DNA content was measured in a separate well and used to normalize the radioactivity. Results are expressed as the mean ± SD of five wells.

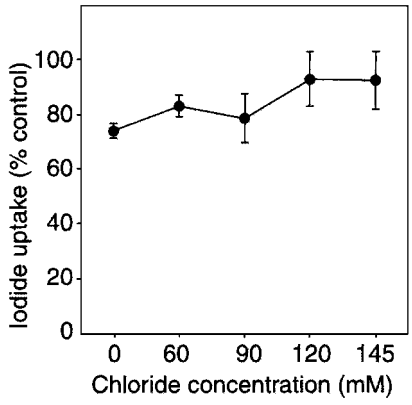

FIG. 3. Effect of chloride on $^{125}I^-$uptake by pendrin. P-COS cells were incubated with 1 mM iodide in various concentration of chloride-containing buffer for 2 h. After incubation, medium was removed, and cells were solubilized and collected for counting. $^{125}I^-$ uptake is expressed as the mean ± SD percentage in five wells against 140 mM chloride.

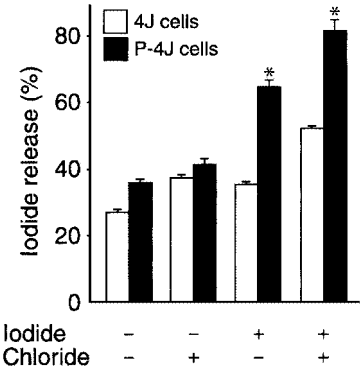

FIG. 4. Iodide efflux by pendrin. CHO cells expressing NIS (4J cells) and both NIS and pendrin (P-4J cells) were initially incubated with 10 μM $Na^{125}I$ in HBSS buffer for 1 h, then cells were washed, and fresh medium containing iodide, chloride, or both was added for 5 min. Radioactivity in the culture supernatant and cells was separately counted. Results are expressed as the percent radioactivity that appeared in culture supernatant (mean ± SD). The *asterisk* denotes statistical significance between P-4J cells and 4J cells.

of accumulating iodide at up to a 2-mM intracellular concentration.

Discussion

In the present report we investigated ion transport by pendrin in mammalian cell systems. To do this, we used COS-7 and CHO cells expressing NIS and/or pendrin as well as rat thyroid FRTL-5 cells. We have used the same cells to study the function of NIS and have shown that physiological characteristics of introduced NIS in COS-7 and CHO cells are very similar to those in thyroid cells (16), suggesting that these techniques are a good model for studying ion transport in the thyroid.

Using these mammalian cells we showed that pendrin is important for iodide efflux. Pendrin transported iodide very efficiently only when high concentrations of iodide existed (>1 mM), and it is considered not to be as important for iodide uptake. However, unlike the results obtained with *X. laevis* oocytes and Sf9 insect cells, its function was independent of chloride. This is the major difference between the present study and the published studies described above. It is possible that the function of pendrin may differ among species, as ion transporters and channels sometimes show an altered function when expressed in different cell types (14–16, 26). A recent study revealed that pendrin can function as a chloride/base exchanger, not only in *X. laevis* oocytes, but also in mammalian cells transfected with pendrin (13). The absence of bicarbonate secretion was also demonstrated in tubules isolated from the pendrin knockout mouse (11). These results indicate that pendrin transports chloride not only in nonmammalian cells, but also in mammalian cells. The mechanism of noncompeting transport of iodide and chloride in this study was not clarified in this study. A high salutation level of chloride and iodide transport by pendrin when expressed on mammalian cell membrane could be involved. In fact, competitive transport of iodide and chloride may not be a suitable model for iodide transport in the thyroid, because the reported chloride concentration in thyroid cells (~16 mM) (30) seems to be too high for iodide to be transported against it.

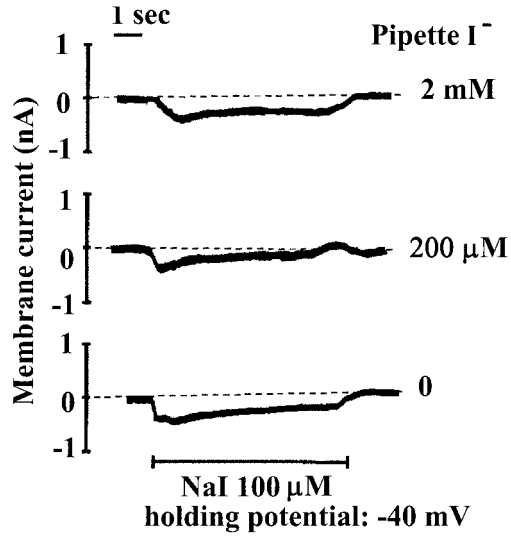

FIG. 5. Activation of transport current with NaI in CHO-4J cells. Changes in membrane current by switching the bathing solution as indicated was measured as previously described (16, 26). The y-axis indicates membrane current induced by translocation of sodium and iodide by NIS. Membrane current was shifted inwardly by iodide and returned after the removal of NaI. The same level of inward current that was observed under conditions of no iodide in the pipette was observed under conditions of 200 μM or 2 mM cytoplasmic iodide.

In this study there was no competitive transport of iodide and chloride in mammalian cells. Thus, neither iodide uptake nor iodide efflux was decreased by chloride in FRTL-5 cells and P-4J cells, respectively. If pendrin competitively transports iodide and chloride even at low concentrations, chloride should displace iodide uptake at least to some extent in FRTL-5 cells, which express endogenous NIS and pendrin (9, 17).

It is not clear how the defect in pendrin results in partial functional abnormalities in the thyroid, because the function of pendrin in the thyroid is not clear. An important function of the thyroid is the organification of iodide within the follicular lumen to synthesize thyroid hormones. Partial functional defects in the organification process and a positive

perchlorate discharge test are characteristic of patients with Pendred syndrome. As pendrin is expressed on the apical membrane (9, 18, 19), and it transports anions (10–13), it is plausible that pendrin mutations result in a defect of iodide transport across the apical membrane of the follicular cells. This explains the organification defect observed in the patients whose iodide uptake at the basolateral membrane by NIS is retained.

Our study strongly supports this idea. Thus, in mammalian cells we showed that pendrin transports iodide only when high concentrations exist (>1 mM), suggesting that pendrin is not responsible for the uptake of iodide from blood, whose iodide concentration is only 1–10 μM. We also showed in this study that pendrin is responsible for iodide efflux. The efflux was stimulated by iodide in the medium. This fact indicated that iodide efflux requires continuous inward flow of iodide to maintain a high concentration of iodide in the cytoplasm. These findings are consistent with the vectorial transport of iodide from the perivascular space to the thyrocyte and then to the follicular lumen. NIS accumulates iodide, which results in an increase in the cytosolic iodide concentration. Iodide may then be exported in a concentration- and membrane potential-dependent manner through pendrin. This result also suggests that pendrin has characteristics of an ion channel rather than a transporter, because a transporter can transport ions at low concentrations against a higher concentration of other ions, whereas the K_m of an ion channel is usually much higher than that of a transporter.

Another interesting piece of evidence is the effect of follicular thyroglobulin (TG) on the expression and function of NIS and pendrin. It was shown that in the absence of TG, NIS expression is maximum, but that of pendrin is minimum (9, 20–22). This mimics the situation in a thyroid follicle where TG accumulation is poor. In such a situation the intracellular concentration of iodide may become higher because of the active uptake by NIS, but poor efflux due to minimal expression of pendrin. Our electrophysiological study revealed that current flow by NIS was still observed under conditions of 2 mM intracellular iodide, indicating that thyroid cells can accumulate iodide up to 2 mM. Low concentrations of accumulated TG significantly induce PDS expression (9), and concentrated iodide within the thyrocyte will now be efficiently transported into follicular lumen by pendrin. This evidence suggests that iodide transport *in vivo* is dynamically regulated by the expression level of NIS and pendrin.

Based on the present results together with previous immunohistochemical studies that demonstrated apical localization of pendrin (9, 18, 19), it seems most likely that pendrin functions as an apical porter of iodide that transports iodide from thyrocytes into the follicular lumen. However, there are other possible explanations for the observed iodide transport in the thyroid. In some ion channels and transporters, function is limited in a rectified manner (30–32). If this is the case for pendrin, then the protein may be an outward rectified transporter of iodide that transports iodide into the cells only when high concentrations of iodide exist, whereas it can transport iodide from the cells even at low concentrations. Previous studies have indicated the existence of iodide channels or iodide/chloride channels responsible for iodide efflux from thyroid cells (27–30). Iodide efflux is considered to be mediated by these iodide/chloride channels and pendrin. The existence of two different mechanisms may explain the complexity and the partial nature of the organification defect seen in Pendred syndrome. Thus, thyroid symptoms are age related, goiter is not always observed, many of the patients are euthyroid, and there is an intrafamilial variability of symptoms (2–4, 33). Many of the ion channels and transporters are closely linked in their function. Therefore, it is possible that an abnormality of one transporter modifies the function of others. For example, pendrin may exchange iodide with an unknown substrate that is subsequently important for maintaining cellular function. Another possible explanation for the diversity of clinical manifestation seen in Pendred syndrome is that the difference in the ability to transport iodide may depend on the changes in amino acid sequence. To prove this, it will be necessary to study the function of pendrin on iodide transport for each mutation found in Pendred syndrome patients.

Acknowledgments

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