Controversies Concerning the Role of Pendrin as an Apical Iodide Transporter in Thyroid Follicular Cells

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Pendred syndrome • Pendrin • SLC26A4 • Thyroid hormone synthesis • Goiter • Hypothyroidism

Abstract
Pendred syndrome is an autosomal recessive disorder defined by sensorineural deafness, goiter and a partial organification defect of iodide. It is caused by biallelic mutations in the multifunctional anion transporter pendrin/SLC26A4. In human thyroid tissue, pendrin is localized at the apical membrane of thyroid follicular cells. The clinical phenotype of patients with Pendred syndrome and the fact that pendrin can mediate iodide efflux in transfected cells suggest that this anion exchanger may be involved in mediating iodide efflux into the follicular lumen, a key step in thyroid hormone biosynthesis. This concept has, however, been questioned. This review discusses supporting evidence as well as arguments questioning a role of pendrin in mediating iodide efflux in thyrocytes.

Introduction
Pendred syndrome (OMIM 274600) is an autosomal recessive disorder defined by sensorineural deafness, goiter, and a partial defect in the organification of iodide [1, 2]. In 1958, Morgans and Trotter demonstrated that patients with Pendred syndrome have a partial iodide organification defect when submitted to a perchlorate discharge test [3], a finding indicating an inefficient synthesis of thyroid hormone. The molecular defect of Pendred syndrome consists in biallelic mutations in the SLC26A4 (Solute Carrier 26A4) gene, also referred to as PDS (Pendred Syndrome) gene, which encodes pendrin, a multifunctional anion transporter [4]. Pendrin has affinity for chloride, iodide, bicarbonate, hydroxide, thiocyanate and formate [5-9]; whether they are all physiological ligands remains unknown. In the thyroid, pendrin has been implicated in playing a role in mediating efflux of iodide into the follicular lumen, the functional unit for the synthesis of thyroid hormones, which are essential regulators of normal development, growth and metabolism [10].

Thyroid hormone biosynthesis

The synthesis of thyroid hormones, thyroxine (T4) and triiodothyronine (T3), requires a normally developed thyroid gland, an adequate iodide intake and a series of regulated biochemical steps in thyroid follicular cells, which form the spherical thyroid follicles [10]. At the...
basolateral membrane of thyrocytes, the Na⁺/K⁺-ATPase generates a sodium gradient that permits the sodium-iodide symporter (NIS) to mediate iodide uptake [11, 12]. Iodide then translocates to the apical membrane and reaches the follicular lumen through the apical membrane. While it has been assumed that iodide moves across the apical membrane primarily because of the electrochemical gradient, studies in frozen sections demonstrated that it is first accumulated in the cytoplasm and only later in the lumen [13], and apical iodide efflux is rapidly accelerated in polarized cells after exposure to thyroid stimulating hormone (TSH) [14]. Electrophysiological studies using inverted plasma membrane vesicles suggested the existence of two apical iodide channels [15], but their molecular identity has not been determined. One of the channels appears to have a high permeability and specificity for iodide (Km ~ 70 µM), while the second channel has a much lower affinity (Km ~ 33 mM). Once in the follicular lumen, iodide is oxidized by the membrane-bound enzyme thyroid peroxidase in the presence of hydrogen peroxide (H₂O₂). Oxidized iodide is then organified into selected tyrosyl residues of thyroglobulin, a very large secreted glycoprotein that serves as the scaffold for thyroid hormone synthesis, and this results in the formation of mono- and diiodotyrosines (MIT, DIT). In the subsequent coupling reaction, which is also catalyzed by thyroid peroxidase, two iodotyrosines are coupled to form either T₄ or T₃. Iodinated thyroglobulin is engulfed by thyrocytes through macro- and micropinocytosis and digested in lysosomes. Released T₄ and T₃ are secreted into the bloodstream at the basolateral membrane primarily because of the electrochemical gradient, studies in frozen sections demonstrated that it is first accumulated in the cytoplasm and only later in the lumen [13], and apical iodide efflux is rapidly accelerated in polarized cells after exposure to thyroid stimulating hormone (TSH) [14]. Electrophysiological studies using inverted plasma membrane vesicles suggested the existence of two apical iodide channels [15], but their molecular identity has not been determined. One of the channels appears to have a high permeability and specificity for iodide (Km ~ 70 µM), while the second channel has a much lower affinity (Km ~ 33 mM). Once in the follicular lumen, iodide is oxidized by the membrane-bound enzyme thyroid peroxidase in the presence of hydrogen peroxide (H₂O₂). Oxidized iodide is then organified into selected tyrosyl residues of thyroglobulin, a very large secreted glycoprotein that serves as the scaffold for thyroid hormone synthesis, and this results in the formation of mono- and diiodotyrosines (MIT, DIT). In the subsequent coupling reaction, which is also catalyzed by thyroid peroxidase, two iodotyrosines are coupled to form either T₄ or T₃. Iodinated thyroglobulin is engulfed by thyrocytes through macro- and micropinocytosis and digested in lysosomes. Released T₄ and T₃ are secreted into the bloodstream at the basolateral membrane, at least in part by the recently identified pendrin at the basolateral membrane [37]. The apical expression pattern and function of this protein remains to be determined, but it is likely that pendrin plays a role in thyroid hormone synthesis.

Expression and putative function of pendrin in the thyroid

In thyroid follicular cells, pendrin is expressed at the apical membrane [37]. The apical expression pattern and pendrin’s ability to transport iodide in Xenopus oocytes [7], suggested that pendrin is one of the entities mediating apical iodide efflux [37]. In addition, the PIOD present in patients with biallelic mutations in the SLC26A4 gene is consistent with a potential role of pendrin in thyroid hormone synthesis. Functional studies of pendrin in Xenopus oocytes initially revealed that pendrin mediates uptake of chloride and iodide in a sodium-independent manner [7]. This was followed by a series of studies in transfected mammalian cells in non-polarized Chinese hamster ovary (CHO) cells
expressing NIS, which mediates iodide uptake in a perchlorate-sensitive manner, the presence of pendrin leads to the efflux of iodide [38]. In time-course experiments measuring iodide release, human embryonic kidney cells transfected with NIS alone demonstrate a slow, time-dependent iodide efflux, whereas cells coexpressing pendrin and NIS exhibit a very rapid efflux of iodide [39]. In polarized Madin-Darby canine kidney (MDCK) cells cultured on semipermeable membranes in a bicameral system, cells stably transfected with NIS display a significant increase in intracellular iodide uptake compared to untransfected MDCK cells [39]. This contrasts with cells expressing NIS and pendrin, which show significant iodide transport into the apical chamber, and, hence, a significant drop in intracellular iodide content. Cells expressing only pendrin show lower intracellular iodide levels than untransfected MDCK cells, but higher levels in the apical chamber; this finding suggests that pendrin facilitates apical release of iodide after its uptake at the basolateral membrane, which is presumably mediated by unspecific chloride channels. Taken together, these observations suggest that pendrin is able to mediate vectorial iodide transport at the apical membrane in polarized cells [39].

Electrophysiological studies using transfected COS-7 cells also reveal that pendrin mediates iodide efflux, and the currents demonstrating iodide efflux are higher with increasing extracellular chloride concentrations [40].

More recent findings in *Xenopus* oocytes demonstrate clearly that SLC26A4 functions as a coupled, electroneutral iodide/chloride, iodide/bicarbonate and chloride/bicarbonate exchanger with a 1:1 stoichiometry [41]. Of particular importance in this context is the fact that iodide is the preferred anion and that SLC26A4 transports iodide in the presence of high chloride [41]. This study also demonstrated luminal iodide secretion in parotis gland ducts, emphasizing that SLC26A4 has a preferential affinity for iodide [41].

The effects of TSH, the main regulator of thyroid cell function and growth, on pendrin expression and function have provided results that are, in part, controversial. In FRTL-5 rat thyroid cells, low doses of TSH do not induce SLC26A4 mRNA determined by Northern blot analyses [37]. This contrasts with findings in the same cell line documenting induction of SLC26A4 mRNA by RT-PCR after exposure to TSH and forskolin [42], as well as findings in the PCCL3 rat thyroid cell line reporting increased expression of pendrin mRNA, determined by RT-PCR, and protein after exposure to high doses of TSH, forskolin and 8-Bromo-cAMP [43]. It is well established, that iodide efflux at the apical membrane is rapidly accelerated by TSH [44-46]. Forskolin-treatment of cells transfected with pendrin results in a rapid increase in membrane insertion and promotes increased iodide efflux [47], an effect that seems to involve the protein kinase A pathway based on studies in PCCL3 rat thyroid cells [48], findings that would be consistent with a role of pendrin in mediating iodide efflux. Another study reported a delayed translocation of pendrin from the cytosol to the plasma membrane after activation of the PKC pathway following exposure of cultured rat thyroid PCC13 cells to insulin [43].

Only a small number of the large number of reported mutations (>170) have been tested functionally in terms of iodide or halide transport [39, 49-51]. The disease-causing mutations result in a complete or partial loss of function in terms of mediating iodide efflux [39, 49, 51, 52], a finding that is also demonstrable in time-course experiments [39]. Fluorometric methods that allow monitoring the intracellular halide content or the pH have been used for selected mutants, and also reveal an absent or partially impaired ability to transport iodide, chloride and bicarbonate [51-53]. Many of the characterized mutations are retained in intracellular compartments, most likely the endoplasmic reticulum [54]. As illustrated in a manuscript by Dossena et al. in this issue, some of the reported sequence alterations are functionally irrelevant and simply reflect polymorphisms in the SLC26A4 gene [55].

**Controversies concerning a physiological role of pendrin in mediating iodide efflux**

Several observations have led to questioning the concept that pendrin plays a physiological role in mediating apical iodide transport in thyroid follicular cells [56, 57]. The fact that some individuals with biallelic mutations in the SLC26A4 gene have no or only a mild thyroidal phenotype [30], indicates that iodide crosses the apical membrane independently of pendrin through another iodide channel or unspecific channels. Thus, the relative importance of pendrin remains uncertain. Knockout mice with targeted disruption of the Slc26a4 gene faithfully replicate the phenotype at the level of the inner ear and continue to provide important insights into the underlying pathophysiology [58-60], but they do not develop a goiter or abnormal thyroid hormone levels [58], even under conditions of iodine deficiency [61, 62], questioning an important role of pendrin in iodide metabolism, at least in the mouse.

It has been emphasized that the intracellular affinity of pendrin for iodide must be substantially higher than for chloride in order to mediate iodide efflux [56]. Although exact affinity constants are not available, it has become
clear that iodide is indeed the preferred anion and that SLC26A4 transports iodide in the presence of high chloride, and that it also mediated luminal iodide secretion in parotis gland ducts [41]. Moreover, the results conducted in polarized cells have been performed under physiological concentrations of iodide and chloride and suggest that pendrin preferentially mediates iodide efflux [39]. Similar conclusions have been drawn from electrophysiological studies with variable extracellular iodide and chloride concentrations [38]. These data also suggest that iodide efflux/chloride influx is more efficient than chloride efflux/chloride influx [38]. It has also been argued that the distinct role of pendrin in the thyroid as a putative iodide/chloride exchanger is intriguing given its function as a chloride/bicarbonate exchanger in the inner ear and the kidney [5, 6, 63, 64]. The differential role may, however, depend on the relative anion concentrations in the respective cells and thyroid cells distinguish themselves by unusually high iodide concentrations due to the expression of NIS [12]; this, and the preference of SLC26A4 for iodide over chloride, should thus result in iodide efflux into the follicular lumen analogous to findings obtained in the parotis ducts [41].

Given that the vast majority of patients with biallelic mutations in the SLC26A4 gene have only a mild or no thyroidal phenotype under normal iodide intake conditions [30], one or several other channels and/or transporters seem to be involved in the apical efflux of iodide into the follicular lumen. Their identity is currently unknown. SLC5A8, originally designated as human apical iodide transporter (hAIT) [65], is not involved in mediating iodide efflux as demonstrated by functional studies in Xenopus oocytes and polarized MDCK cells [66]. Interestingly, mice with targeted disruption of the CLCN5 chloride channel, which is localized at the apical membrane of thyrocytes, develop a thyroidal phenotype that is reminiscent of Pendred syndrome with goiter formation and decreased iodide organification [67]. In humans, CLCN5 is mutated in the X-linked recessive Dent’s disease (hypocalcic nephrolithiasis, proteinuria/aminoaciduria, glycosuria; OMIM 300008), which is not associated with a thyroidal phenotype. Further functional studies are needed to characterize the potential role of CLCN5 in terms of iodide transport. Interestingly, in thyroid tissue from a patient with Pendred syndrome, the absence of pendrin was accompanied by increased CLCN5 expression [68], and it has been speculated that this may compensate for apical iodide efflux.

It is noteworthy that studies in the Slc26a4-/- knockout mouse have shown that the transepithelial potential and the pH of thyroid follicles are reduced [69], suggesting that pendrin-mediated bicarbonate efflux into the follicle and chloride uptake into thyrocytes is diminished. Hence, it is conceivable that a change in intrafollicular pH could result in impaired organification of iodide, for example by reducing the efficiency of thyroid peroxidase.

**Conclusion**

Patients with Pendred syndrome have a mildly impaired iodide organification defect and they can present with goiter and more rarely with hypothyroidism. Based on studies in frog oocytes and mammalian cells, it is clear that pendrin has a higher affinity for iodide than for chloride and bicarbonate, and that it is able to mediate apical iodide efflux in polarized cells. Moreover, pendrin membrane abundance and iodide efflux appear to be stimulated by TSH in rat thyroid cells. Apical iodide efflux is, however, also possible in the absence of pendrin implicating the presence of at least one additional iodide transporting entity. In addition, it is conceivable that the impaired organification could result from an alteration in follicular pH.

Addressing these persisting controversies should permit to better establish the role of pendrin in the pathophysiology of the thyroid gland in the near future.

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