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Pendrin: the Thyrocyte Apical Membrane Iodide Transporter?

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Pendrin • Thyroid • Iodide transport • Experimental model

Abstract
In the thyroid, the transport of iodide from the extracellular space to the follicular lumen requires two steps: the transport in the cell at the basal side and in the lumen at the apical side. The first step is mediated by the Na^+/I^- symporter (NIS). In most reviews and textbooks, the second step is presented as mediated by pendrin. In this review, we analyze this assumption. There are several arguments supporting the concept that indeed pendrin plays an important role in thyroid physiology. However, biochemical, clinical and histological data on the thyroid of a patient with Pendred syndrome do not suggest an essential role in iodide transport, which is corroborated by the lack of a thyroid phenotype in pendrin knockout mice. Experiments *in vivo* and *in vitro* on polarized and unpolarized cells show that iodide is transported transport of iodide at the apex of the thyroid cell. Moreover, ectopic expression of pendrin in transfected non-thyroid cells is capable of mediating iodide efflux. It is concluded that pendrin may participate in the iodide efflux into thyroid lumen but not as the unique transporter. Moreover, another role of pendrin in mediating Cl^-/HCO_3^- exchange and controlling luminal pH is suggested.

Introduction

The synthesis of thyroid hormones in the thyroid gland involves several steps: the uptake of iodide by the sodium/iodide symporter NIS, the release of this iodide in the follicular lumen, the oxidation of this iodide by thyroperoxidase using H_2O_2 generated by the DUOXES (dual oxidases), its covalent binding to the tyrosyl groups of thyroglobulin and the final oxidative coupling of the iodotyrosines within thyroglobulin into iodothyronines. While the other proteins involved in this metabolism are well known, the identity of the apical membrane transporter has not been unambiguously established. Several arguments and authors suggest that pendrin, which belongs to the SLC26 family of anion exchangers (SLC26A4), could be this transporter [1] but some other authors disagree [2, 3]. Pendrin gene biallelic inactiva-

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tion leads to the Pendred syndrome. This syndrome is characterized by deafness of all the patients, and relatively late goiter and hypothyroidism in the majority of them [4].

We shall discuss the possibility that the thyroid defect is due to a deficiency of apical iodide transport by pendrin.

Arguments for a role of pendrin in thyroid physiology

There is no doubt that pendrin plays a role in thyrocyte physiology. It is highly expressed in human and mouse thyroids [5, 6]. In fact, pendrin mRNA is one of five most abundant transcripts coding for transmembrane proteins expressed in the human thyroid. In human thyrocytes and in the rat thyroid cell line PCCl3, pendrin mRNA is upregulated by the differentiating TSH and its intracellular signal cAMP [7, 8]. Conversely, it is downregulated by the dedifferentiating EGF in human thyrocytes [5]. Its thyroid expression is decreased in papillary carcinomas and suppressed in the totally dedifferentiated anaplastic carcinomas (Hébrant A, personal communication).

A proportion of patients with Pendred syndrome develop hypothyroidism and goiter [1]. However, no thyroid phenotype has been reported in mice KO for pendrin, even with a low iodine diet [9].

Lessons from the Pendred syndrome patients

Defects in the iodide transporter NIS or in the enzymes essential for thyroid hormone synthesis (e.g. TPO, DUOX2, DUOXA2) or in the main controlling signal (TSH and the TSH receptor) give rise to hypothyroidism at birth, an in case of the enzymes, to rapid goiter induction. In contrast Pendred patients do not develop early hypothyroidism or goiter. This and the phenotype of KO mice suggest that pendrin is not an essential factor in the process of hormone synthesis and therefore the unique, necessary apical iodide transporter of iodide. However, one can always argue that even in the absence of the transporter enough iodide might get through the apical membrane especially if iodide supply was high.

A goiter from a patient with Pendred syndrome has been investigated [10]. First, iodide uptake in methimazole-blocked thyroid slices was similar in the goiter tissue and in normal controls, which does not suggest that one iodide compartment (the follicular lumen) is missing in the Pendred thyroid unless TSH compensation had increased NIS expression and thereby the cellular content. Second, the release of iodide from precharged methimazole-blocked slices was similar in Pendred and normal tissues, whereas we could have expected a faster release from the Pendred tissue if its iodide content was stored only in the intracellular compartment.

The pathology of a goiter from a patient with Pendred syndrome gives another view of the problem [10]. In a congenital defect, all the defective cells behave similarly. In the Pendred goiter (no pendrin expression), three types of zones were identified:

1) normal zones with iodinated thyroglobulin in the follicular lumen, which shows that in these follicles iodide has normally accessed the lumen.

2) zones in which no iodinated thyroglobulin was present in the follicular lumen. In these zones, DUOX and TPO were located intracellularly and evidence of oxidative stress was seen.

3) zones of follicular disruption with evidence of cell necrosis, apoptosis and compensatory proliferation.

From these data, it was deduced that the gland compensates (through the use of another channel such as CLC5, CFTR or SLC26A7) the lack of pendrin. However, impaired function (intracellular trafficking) led to intracellular accumulation of DUOX and TPO, resulting in oxidative stress (due to generation of O_2^- and H_2O_2 in the cell) and ultimately in cell death by apoptosis and necrosis. Of course, this suggestion represents the kinetic interpretation of one picture of the gland at one time! Nevertheless, this picture is very different from the one seen with defects of the other thyrocyte-specific genes such as NIS, DUOX2, DUOXA2, TPO and thyroglobulin where all cells are affected and display features of hyperstimulation which leads to goiter. The Pendred thyroid histology rather suggests a defect leading to a progressive deterioration of the cells with ultimate death. Furthermore, there are no arguments for a defect in iodide uptake. The defect rather seems to occur at the level of iodide oxidation and organification (positive perchlorate test: discharge of radioiodide after perchlorate administration).

In conclusion, all these *in vivo* data show that indeed pendrin is an important protein in thyroid physiology but do not suggest an essential role for this protein in thyroid iodide transport.

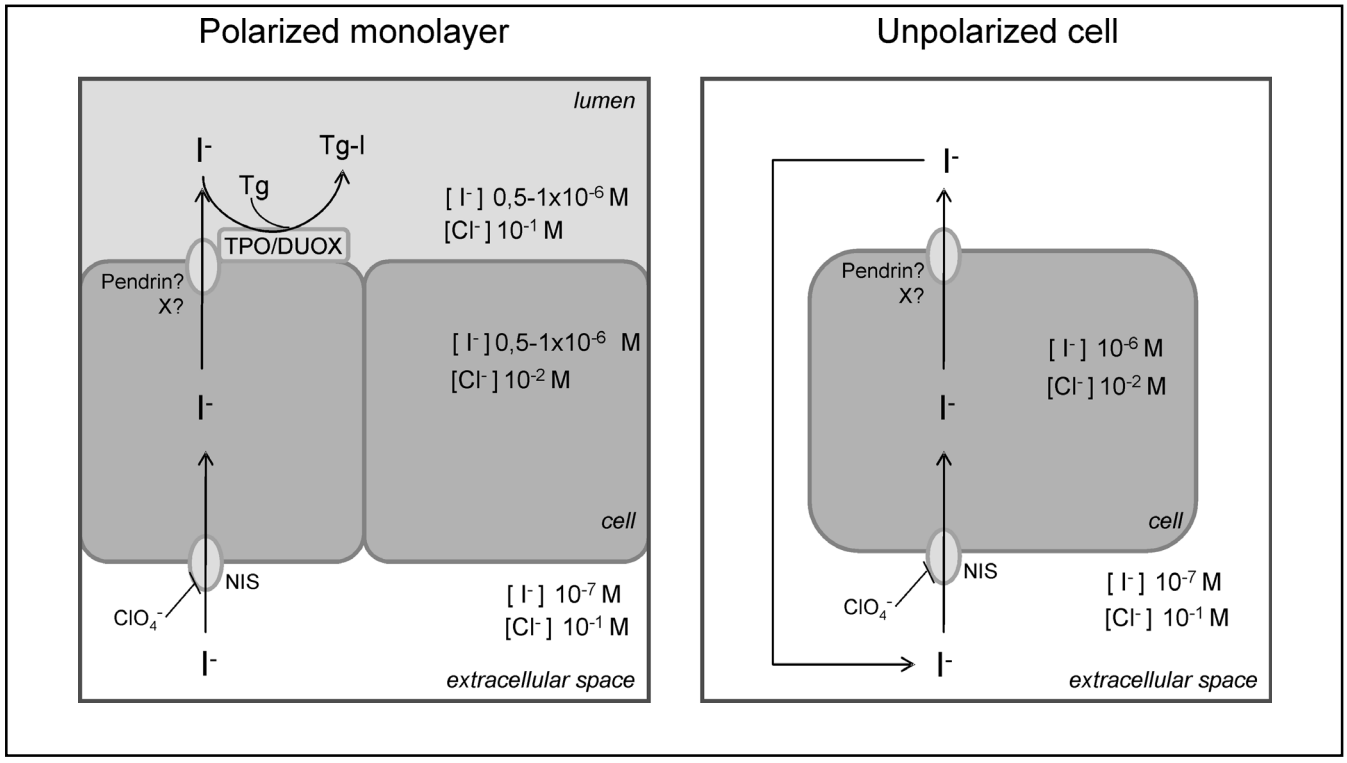

Fig. 1. Scheme of iodide transport through a polarized thyrocyte epithelium (left panel) and in unpolarized cells (right panel). The polarized epithelium represents a three-compartment model while unpolarized cells expressing NIS and pendrin (or any other candidate protein transporting iodide) represent a two-compartment model. The estimated concentrations of iodide and chloride in each compartment are given. If I^- is not oxidized and organified, it can return to the basolateral space through the paracellular pathway due to a concentration and an electrical gradient of about 10 mV [13].

Iodide uptake in the thyroid

When analyzing the biochemistry of iodide uptake by the thyroid, consideration of the cell physiology and the *in vivo* conditions of this uptake is warranted (Fig. 1). First of all, while the importance of NIS for iodide uptake at the basolateral membrane is unambiguously established, the transport step at the apical membrane remains ill-defined: carrier-mediated vs non-selective channels. Several arguments suggest that, indeed, the cell to lumen efflux is not immediate and may, under some circumstances, limit the availability of iodide in the lumen. Experiments on the kinetics of radioiodide uptake in dog thyroid slices in which iodide organification was blocked showed that the kinetics could best be accounted for by a sequential three-compartment model. These compartments were assumed to be the extracellular, the cellular and the luminal fluid spaces. Using this model, it was shown that iodide first penetrated in the cell, then moved with some delay into the follicular lumen [11]. There was no instantaneous equilibrium. More physiologically, the group of Wolman has shown by radioautography of radioiodide-injected mice an initial cellular uptake later followed by equilibrium with the follicular lumen. These very clean results were obtained thanks to the use of hypophysectomized (i.e. TSH deprived) mice whose thyrocyte metabolism was therefore slowed down [12].

Finally, in well designed beautiful experiments, Nilsson's group set up *in vitro* polarized pig thyrocyte monolayers which were used to measure radioiodide uptake by the cells and its transport from a basal compartment facing the basolateral membrane to the cell and to an apical compartment bordering the apical membrane [13, 14]. Stimulation of the cell by TSH in the basal compartment led to an increased efflux from the cell to the apical compartment and a decrease of radioiodide within the cells. Thus, there exists a transport at the apical membrane and this transport is activated by TSH [13].

Several *in vitro* experimental models have been used for the study of iodide transport by the thyrocytes, from

the physiological thyroid slices and polarized thyrocyte monolayers to the less physiological, but convenient, primary unpolarized cultures of thyrocytes and of rat thyroid cell lines (PCCl3, FRTL5). Isolated particulate vesicles or thyroid membrane proteins reconstituted in proteoliposomes represent an even less physiological but simple and more precise model [15-17]. All these models investigate the bona fide thyrocyte iodide exporter whatever it is. On the contrary, non-thyroid cell lines expressing NIS and other postulated iodide transporters are not physiological but they allow testing directly the properties of candidate proteins. Interestingly, in FRTL5 cell cultures and primary cultures of unpolarized dog thyrocytes, iodide efflux can be stimulated efficiently by intracellular Ca^{++} and barely by cAMP, whereas in primary cultures of polarized pig thyrocytes, the efflux is only stimulated by cAMP [18-20]. This suggests a species-dependent regulation of the apical iodide transporter, as for H_2O_2 generation [21].

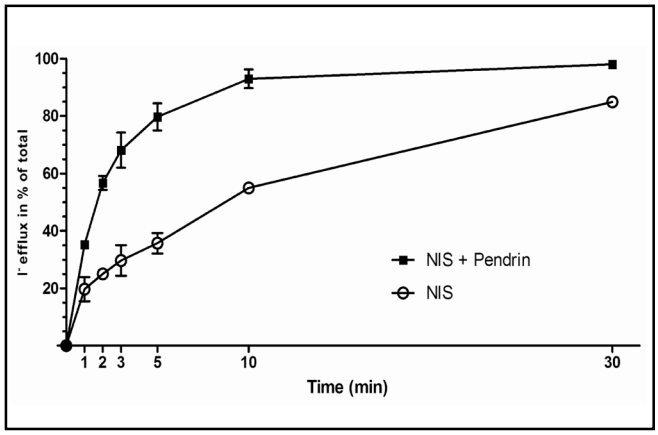

Fig. 2. Kinetics of radioiodide efflux in HEK-293T cells expressing NIS alone or in combination with pendrin. Cells were transfected with Fugene 6 according to the manufacturer's instructions. Seventy-two hours later, the cultures were preincubated for 45 min with 10^{-7} M iodide (1 μCi/ml $^{125}I^-$) after which efflux was measured in the presence of 1 mM $NaClO_4$.

Model of iodide transport

Two questions arise: is pendrin able to transport iodide under physiological conditions? If yes, is pendrin the main or sole thyrocyte apical membrane iodide transporter? Before analyzing these questions, we shall first consider the physiological model of iodide transport (Fig. 1). Iodide is transported vectorially across the follicular epithelial cell: NIS, inserted within the basolateral membrane in parallel with the Na^+/K^+-ATPase, cotransports in an obligatory fashion two Na^+ with one I^- [22] and thus actively concentrates iodide inside the thyrocyte from the medium, at least by a factor 10. This represents a classical secondary active transport mechanism, driven by the electrochemical gradient for Na^+ across this membrane, and is therefore progressively blocked by ouabaïn. Intracellular iodide then exits at the apical membrane either by pendrin or by another transporter, driven by the iodide electrochemical potential gradient across the apical membrane. If pendrin mediates the electroneutral exchange of one Cl^- for one I^-, in thyrocyte monolayer cultures, net uptake should then stop when cells and apical medium reach equilibrium.

What happens during a perchlorate discharge test or in case of an organification defect? In the latter case, one hypothesis is that iodide that has reached the intrafollicular lumen can exit through the paracellular shunt pathway through the tight junctions, driven by the transepithelial potential difference (about 10 mV, lumen negative) as well as by the sizeable transepithelial concentration difference. When perchlorate is given after radioiodide, the latter can still exit 1) through the normal apical pathway and return from the cell to the extrafollicular side, and 2) also through the junctional pathway, as in organification defects. Efflux through unspecific anion channels of the basolateral membrane could also contribute to the iodide discharge, but no such anion channel has been reported so far. Thus, the thyroid follicle can be experimentally modeled by polarized cells with three compartments.

In polarized cells, such as in the slices where the equilibrium ratio of iodide taken up vs medium radioiodide is 5 to 10 but can reach 50 to 80, it is assumed that cellular and luminal concentrations are similar with slightly lower concentrations in the lumen as long as iodide oxidation and organification occur [11, 12]. However, in unpolarized PCCl3 cells, where both uptake and efflux converge into or from one unique compartment, the ratios obtained are of the order of 3 to 20.

As pendrin is proposed to exchange I^-/Cl^- in a 1/1 electroneutral way, the concentrations of both ions in the different compartments, as well as the affinity of the exchanger for each anion should be considered. Iodide in the extracellular fluid is generally around 10^{-7} M but after heavy iodide-containing medication can rise up to 10^{-4} M. The Km of NIS is about 3.10^{-5} M for I^- and for Na^+ 28 mM [22]. The Km of pendrin is 2.5 mM for Cl^- but

has not been determined for I^- [23]. Thus with "physiological" concentrations of iodide of 10^{-7} M outside the cell (i.e. in the plasma) one can calculate a concentration of $0.5\text{-}1.10^{-6}$ M or even higher (when NIS is stimulated) in the cell and in the lumen. This should be compared with chloride concentrations of 10^{-1} M outside and 10^{-2} M in the cell. Thus within the cytoplasm, I^- and Cl^- would compete for binding to pendrin at concentrations differing by four orders of magnitude. This makes electrophysiological measurements carried out at 10^{-3} M for both ions physiologically irrelevant. Thus, if pendrin carries equal fluxes of I^- and Cl^- at concentrations that differ by a magnitude of at least 10^4, this implies that its affinity for these anions differ by the same order of magnitude.

Is pendrin able to transport iodide under physiological conditions?

Measurements of radioiodide efflux from pre-labeled non-thyroid cells (JEG-3, HEK-293T, COS) expressing NIS, with or without pendrin, provide a clear answer to this question. One may first ask which protein mediates iodide efflux in cells expressing NIS and no other specific thyrocyte membrane protein, in particular no pendrin. The slow efflux observed under these conditions likely occurs through non selective anion channels such as the volume-sensitive anion channels or other unspecific transporters not necessarily expressed in the thyrocytes. If pendrin effectively mediates iodide efflux, the pendrin-expressing cells will release iodide much faster than the NIS without pendrin cells, as indeed was observed by both P. Kopp's group [24] and our group (Fig 2). Moreover, this effect does not take place for pendrin inactivated by mutation [24]. Similarly, in human thyrocyte cell lines expressing an inactive pendrin mutant, the uptake of radioiodide by the cells is higher and slower as expected for cells with lower efflux [25, 26]. However, while iodide efflux was acutely stimulated by TSH and forskolin in the pig thyrocyte monolayers [13], this does not appear to hold for «non-thyrocyte» reconstituted polarized systems such as the MDCK monolayers expressing both NIS and pendrin, even though these proteins are correctly addressed to their respective membrane domains in this system. Thus, while there is no doubt that pendrin can transport iodide under physiological ionic conditions, the acute TSH stimulation of iodide efflux at the apical membrane of pig thyrocytes remains unexplained at the molecular level.

Conclusions

The available evidence thus suggests: that pendrin is an important thyroid differentiating protein; that pendrin is able to transport iodide under physiological ionic conditions but this role appears *in vivo* dispensable; that pendrin cannot be the sole apical thyrocyte iodide transporter.

Other roles of pendrin in thyrocyte physiology should be considered. Pendrin is mostly considered in other organs (cochlea, kidney, airway mucosae) as a Cl^-/HCO_3^- exchanger [9, 27, 28]. On the other hand, unpublished work from our laboratory shows the essential importance of pH in H_2O_2 generation. We would therefore suggest that a major role of pendrin in the thyroid is the exchange of intracellular HCO_3^- for luminal chloride, thus increasing luminal fluid pH. Indeed, in pendrin KO mice, even in the absence of overt thyroid dysfunction, the luminal pH is decreased [9, 29].

Abbreviations

TSH (thyrotropin); EGF (epidermal growth factor); cAMP (cyclic adenosine 3',5'-monophosphate); DUOX (dual oxidase); TPO (thyroperoxydase); NIS (Na^+/I^- symporter).

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