The Controversial Role of Pendrin in Thyroid Cell Function and in the Thyroid Phenotype in Pendred Syndrome

7

Peter Kopp, Aigerim Bizhanova, and Liuska Pesce

Abstract

Thyroid hormones are essential for normal development, growth and differentiation of numerous tissues, and metabolic regulation. Structurally, they are unique because they contain iodine. Their synthesis in thyroid follicles thus requires a sufficient nutritional iodide intake, transport into the thyroid cells, and efflux into the follicular lumen where the actual biosynthesis occurs. Historically, Pendred syndrome has been defined by the triad of sensorineural deafness/hearing impairment in combination with goiter and an abnormal organification of iodide. After the identification of the molecular basis of Pendred syndrome, which is caused by biallelic mutations in the *SLC26A4/PDS* gene, functional studies revealed that pendrin is a multifunctional anion exchanger with affinity, among others, for chloride, iodide, and bicarbonate. This observation, together with the demonstration of pendrin protein expression at the apical membrane of thyrocytes, led to the hypothesis that pendrin might be involved in the efflux of iodide into the follicular lumen. Several experimental observations do indeed support a potential role of pendrin in mediating iodide efflux. However, iodide efflux is also possible in the absence of pendrin, and *Slc26a4 −/−* knockout mice do not have a thyroidal phenotype. These findings indicate that other exchangers or channels have a redun-

P. Kopp (✉)
Division of Endocrinology, Metabolism and Molecular Medicine, Department of Internal Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611, USA
e-mail: p-kopp@northwestern.edu

A. Bizhanova
Harold Washington College, 30 E Lake St, Chicago, IL 60601, USA
e-mail: abizhanova@ccc.edu

L. Pesce
Stead Family Department of Pediatrics, Division of Pediatric Endocrinology and Diabetes, University of Iowa Carver School of Medicine, Iowa City, IA 52246, USA
e-mail: liuska-pesce@uiowa.edu

S. Dossena, M. Paulmichl (eds.), *The Role of Pendrin in Health and Disease*,
DOI 10.1007/978-3-319-43287-8_7

dant or perhaps predominant function. A potential candidate is anoctamin 1 (ANO1/TMEM16A), a calcium-activated anion channel. Anoctamin is also expressed at the apical membrane of thyrocytes, and it has affinity for iodide.

Further studies are needed in order to define the relative physiological role of pendrin and anoctamin in mediating iodide efflux, to characterize their affinity for iodide, and to analyze their species-specific expression pattern.

7.1 Introduction

Thyroid hormones are essential for normal development, differentiation, and metabolism of the majority of organs. Their synthesis requires intact follicles, which form the functional units of the gland, several regulated biochemical steps, and an adequate nutritional iodide uptake (Kopp 2012; Pesce and Kopp 2014). At the basolateral membrane of thyroid follicular cells, iodide uptake is mediated by the sodium-iodide symporter (NIS) (Portulano et al. 2014). The function of NIS is dependent on the sodium gradient generated by the Na^+/K^+-ATPase (Fig. 7.1), and a constitutively active potassium channel consisting of the KCNQ1 and KCNE2 subunits, which promotes K^+ efflux (Frohlich et al. 2011; Roepke et al. 2009). At the apical membrane, iodide enters the follicular lumen where the actual thyroid hormone synthesis occurs. Within the follicular lumen, iodide is oxidized by the membrane-bound heme enzyme thyroid peroxidase. The oxidation requires the presence of hydrogen peroxide (H_2O_2), which is generated by the dual oxidase (DUOX) system (Moreno and Visser 2007). Subsequently, oxidized iodide is organified into defined tyrosyl residues of thyroglobulin, which serves as the scaffold for thyroid hormone synthesis. Thyroglobulin is a heavily glycosylated and very large protein (330 kDa) that forms dimers (Di Jeso and Arvan 2016). In a first step, referred to as *organification*, the iodination leads to the formation of mono- and diiodotyrosines (MIT, DIT). A donor and an acceptor iodotyrosine are then fused in the coupling reaction, which generates T4 or T3. Iodinated thyroglobulin is digested by several endopeptidases, both in the follicular lumen and after uptake into the cells through macro- and micropinocytosis (Di Jeso and Arvan 2016). T4 and T3 are secreted into the bloodstream at the basolateral membrane, at least in part by the monocarboxylate transporter MCT8 (Di Cosmo et al. 2010). Remarkably, MIT and DIT are sorted and then deiodinated by an intracellular iodotyrosine dehalogenase (DEHAL1), which permits to recycle the released iodide into the follicular lumen (Moreno et al. 2008; Kopp 2012).

7.2 How Does Iodide Enter the Follicular Lumen?

In electrophysiological studies performed more than two decades ago with inverted thyroid membrane vesicles, Golstein et al. suggested that iodide efflux is mediated by two distinct channels (Golstein et al. 1992). The first one, referred to as the

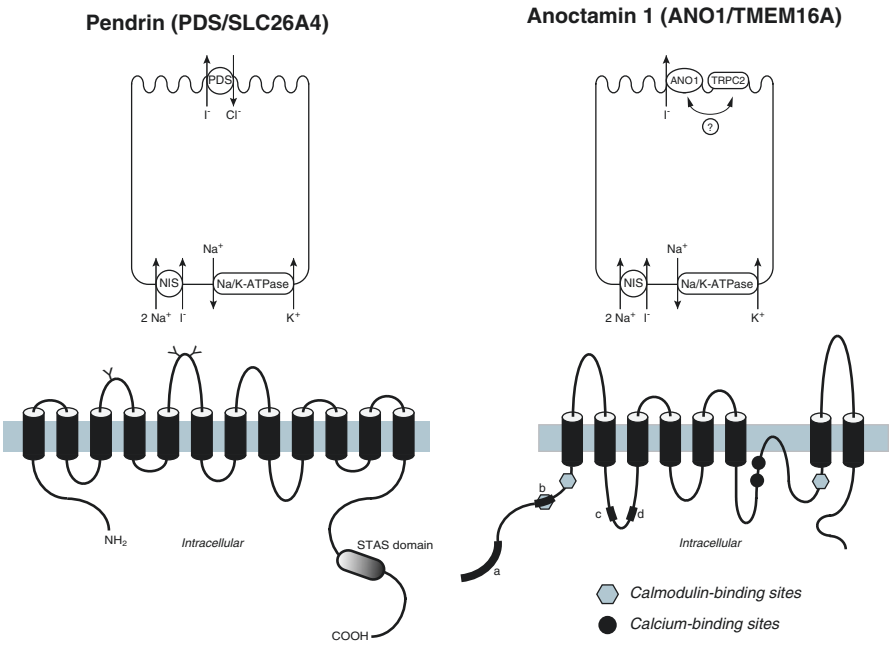

Fig. 7.1 Cellular localization (*top*) and putative structure (*bottom*) of pendrin (PDS/SLC26A4) and anoctamin 1 (ANO1/TMEM16A). Pendrin contains a carboxyterminal STAS (sulfate transporter and antisigma factor antagonist) domain that contains a protein kinase A phosphorylation site. The predicted calmodulin- and calcium-binding sites of ANO1 are indicated. The sodium-iodide symporter (NIS) transports iodide into the follicular cells at the basolateral membrane against the electrochemical gradient. NIS is dependent on the sodium gradient generated by the Na^+/K^+-ATPase. At the apical membrane, pendrin is thought to function as a iodide/chloride exchanger, and anoctamin is a channel that may function in conjunction with other channels such as the transient receptor potential channel 2 (TRPC2)

iodide channel, exhibited a high permeability and specificity for iodide with an approximate Km of 70 μM. The second channel was found to be about fourfold more permeable to iodide than chloride with a Km of about 33 mM. The authors postulated that the iodide channel is restricted to the apical membrane and transports iodide from the cytosol into the colloid space, whereas the second one may mediate predominantly chloride transport under physiological conditions. So far, the molecular identity of these two conductances, which could be anion channels or transporters, has however not been identified. Currently, two candidates have been considered: pendrin and anoctamin (Silveira and Kopp 2015) (Table 7.1 and Fig. 7.1). Chloride channels such as the cystic fibrosis transmembrane conductance regulator (CFTR) and the voltage-gated chloride channel 5 (CLCN5) are also expressed in thyroid follicular cells (Li et al. 2010; van den Hove et al. 2006). While they have affinity for iodide, they are not thought to play a role in mediating iodide efflux under physiological conditions. SLC5A8, a homologue of NIS, which was initially called human apical iodide transporter (hAIT) (Rodriguez et al. 2002), is

Table 7.1 Observations for and against a physiological role of pendrin and anoctamin as mediators of iodide efflux in thyroid follicular cells

Pendrin (SLC26A4)	Anoctamin 1 (ANO1, TMEM16A)
Findings supporting a physiological role of pendrin	*Findings supporting a physiological role of anoctamin*
Patients with Pendred syndrome have a partial iodide organification defect	ANO1 is abundantly expressed at the apical membrane of thyroid follicular cells
Patients with Pendred syndrome can develop a goiter	ANO1 expression is regulated by TSH
Patients with Pendred syndrome can present with congenital or acquired hypothyroidism	ANO1 can be stimulated by calcium
Pendrin is a multifunctional anion exchanger abundantly expressed at the apical membrane of thyroid follicular cells	ANO1 mediates efflux of iodide in transfected cells
Pendrin mediates iodide transport in transfected non-polarized cells	Inhibition or siRNA knockdown of ANO1 decreases iodide efflux from thyroid cells
Pendrin mediates vectorial iodide efflux in transfected polarized cells	ANO1 splice variants expressed in the thyroid have a higher affinity for iodide than chloride
Mutations of pendrin lose the ability to mediate iodide efflux	Studies in oocytes have demonstrated that ANO1 is a calcium-activated chloride channel with a preference for iodide over chloride
Pendrin has a higher affinity for iodide than for chloride	
TSH mediates rapid translocation of pendrin to the apical membrane and increases iodide efflux	
Membrane abundance and half-life increased under conditions of iodide excess	
Findings possibly questioning a primary physiological role of pendrin	*Findings possibly questioning a primary physiological role of ANO1*
TSH does not regulate *PDS/SLC26A4* gene expression	ANO1 may function in conjunction with other channels such as the transient receptor potential channel 2 (TRPC2)
Slc26a4 −/− mice do not display a thyroidal phenotype *This could, however, simply reflect a species difference*	In transfected HEK293 cells expressing pendrin, the iodide efflux is higher compared to cells expressing ANO1 (Fig. 7.2)
Pendrin has a distinct role as an exchanger of chloride and bicarbonate in other tissues such as the kidneys and inner ear	

siRNA small interfering ribonucleic acid, *HEK293* human embryonic kidney cell line 293

clearly not involved in apical iodide efflux as formally demonstrated in functional studies in oocytes and polarized MDCK cells (Paroder et al. 2006).

Thyroid-stimulating hormone (TSH) rapidly activates the efflux of iodide efflux at the apical membrane through the protein kinase A and C pathways (Weiss et al. 1984; Nilsson et al. 1990, 1992; Iosco et al. 2014). These pathways may be differentially regulated depending on the physiological status.

7.3 Pendrin

The classic phenotype of the autosomal recessive Pendred syndrome (OMIM #274600) consists of sensorineural deafness associated with inner ear malformations, especially enlarged vestibular aqueduct (EVA), goiter, and a partial iodide organification defect (PIOD) (Pendred 1896; Morgans and Trotter 1958; Bizhanova and Kopp 2010). Pendred syndrome is caused by biallelic (homozygous and compound heterozygous) mutations in the *PDS/SLC26A4* gene (Everett et al. 1997). Functionally, pendrin belongs to the SLC26 family of multifunctional anion transporters (Alper and Sharma 2013), and it was found to have affinity for iodide (Scott et al. 1999).

Because of the human phenotype with a partial iodide organification defect (PIOD), goiter, and in a subset of subjects congenital or acquired hypothyroidism (Gonzalez Trevino et al. 2001; Ladsous et al. 2014), the expression of pendrin at the apical membrane of thyroid follicular cells (Royaux et al. 2000) (Fig. 7.1), and its affinity for iodide (Scott et al. 1999), it is a plausible candidate for mediating iodide efflux at the apical membrane.

Initial studies in *Xenopus* oocytes demonstrated that pendrin is able to mediate uptake of anions such as chloride and iodide in a sodium-independent manner (Scott et al. 1999). In transfected unpolarized mammalian cells, pendrin was then shown to mediate iodide release (Yoshida et al. 2002). Next, studies performed in polarized cells expressing NIS at the basolateral membrane and pendrin at the apical membrane in a bicameral system demonstrated that pendrin can mediate vectorial iodide efflux at the apical membrane (Gillam et al. 2004). Importantly, more recent studies performed in oocytes have shown that pendrin functions as a coupled, electroneutral iodide/chloride, iodide/bicarbonate, or chloride/bicarbonate exchanger with a 1:1 stoichiometry, and that it has a preferential affinity for iodide, even in the presence of high chloride concentrations (Shcheynikov et al. 2008). Moreover, it has been shown that pendrin is expressed at the apical membrane of parotid gland ducts where it can mediate luminal iodide secretion (Shcheynikov et al. 2008).

Some of the more than hundred naturally occurring mutations have been tested functionally, mainly after transfection into heterologous mammalian cells. Disease-causing mutations result in a complete or partial loss in iodide efflux (Gillam et al. 2004; Taylor et al. 2002; Pera et al. 2008; Dossena et al. 2009, 2011), and many mutated proteins are retained in intracellular compartments such as the endoplasmic reticulum secondary to misfolding (Rotman-Pikielny et al. 2002). The human phenotype, which is characterized by goiter development under conditions of scarce iodide intake (Gonzalez Trevino et al. 2001), as well as the PIOD, is suggestive for a physiological role of pendrin in mediating or participating in iodide efflux in humans. In contrast, however, *Slc26a4 −/−* mice do not develop a goiter or abnormal thyroid hormone levels, even under conditions of iodine deficiency (Calebiro et al. 2011; Iwata et al. 2011). Although this has been used as an argument against a physiological role of pendrin in mediating iodide efflux in thyrocytes (Twyffels

et al. 2011), it is currently unclear whether this simply reflects a species difference (Bizhanova and Kopp 2011).

Iodide efflux at the apical membrane is rapidly accelerated by TSH (Nilsson et al. 1990, 1992; Weiss et al. 1984). Stimulation of the protein kinase A pathway in rat thyroid cells results in a rapid increase in membrane insertion of pendrin and an increased iodide efflux (Bizhanova et al. 2011; Pesce et al. 2012). Stimulation with forskolin increases the membrane insertion of pendrin in PCCL3 rat thyroid cells, presumably through activation of a protein kinase A site in the intracellular carboxyterminus (Bizhanova et al. 2011). Deletion or targeted mutation of the protein kinase A site residing in the intracellular carboxyterminal tail containing the so-called STAS (sulfate transporter and antisigma factor antagonist) domain results in decreased basal function and membrane insertion of pendrin (Bizhanova et al. 2011). In addition, the protein kinase A site mutation (T717A) is partially functional, but it has a mitigated response to forskolin (Bizhanova et al. 2011). Trafficking of murine pendrin to the apical membrane is also activated by cAMP in microperfused mouse cortical collecting duct (CCD) and in polarized renal opossum kidney proximal tubule (OKP) cells via phosphorylation of S49 in the aminoterminal intracellular domain (Azroyan et al. 2012). In contrast to the rapid translocation mediated by the protein kinase A pathway, stimulation of the protein kinase C pathway in rat thyroid cells appears to result in a delayed translocation of pendrin to the plasma membrane (Muscella et al. 2008). Interestingly, rat thyroid cells incubated with supraphysiological amounts of iodide show an increased abundance of pendrin at the plasma membrane, the half-life of pendrin increases, and the efflux of iodide is enhanced (Calil-Silveira et al. 2016). These findings suggest that pendrin could have a role in mediating iodide efflux under conditions of iodide excess.

Under conditions of normal or abundant iodide intake, goiter development is unusual (Sato et al. 2001), but goitrous congenital and overt hypothyroidism developing later in life can be present in patients with Pendred syndrome (Gonzalez Trevino et al. 2001; Ladsous et al. 2014). In a study from Northern France, a region which has a normal to marginal iodide intake, Ladsous et al. characterized the thyroid phenotype in patients with Pendred syndrome and non-syndromic EVA (Ladsous et al. 2014). Fifteen out of the 19 patients with Pendred syndrome (79 %) presented with a goiter. Fifteen (79 %) subjects had hypothyroidism: 6/15 had congenital hypothyroidism, 5/15 had overt hypothyroidism, and 4/15 had subclinical hypothyroidism. Ten out of 16 (63 %) of these patients showed abnormal iodide organification as determined by a perchlorate test, a test that is unfortunately poorly standardized and subject to exogenous (e.g., iodine intake) and endogenous (e.g., autoimmune thyroid disease) modulators. The study by Ladsous et al. clearly demonstrates that there is a relatively wide spectrum in the thyroid phenotype among patients with Pendred syndrome, suggesting that it is influenced by genetic and environmental modifiers, including nutritional iodine intake (Ladsous et al. 2014). Intriguingly, biallelic mutations in the *SLC26A4* gene have also been identified in two patients with thyroid hypoplasia and congenital hypothyroidism from two unrelated families (Kühnen et al. 2014). The mutations found in these subjects have been previously identified in patients with the classical form of Pendred syndrome or

familial EVA (Kühnen et al. 2014; Kopp 2014). The reasons why these two patients developed thyroid hypoplasia, rather than a goitrous phenotype, are unclear. It has been speculated that either the retained misfolded proteins or an increased production of free radicals in response to sustained stimulation by TSH could have a toxic effect leading to cell death, or that the hypoplastic phenotype requires the presence of additional modifying (genetic) factors (Kopp 2014; Kühnen et al. 2014).

7.4 Anoctamin

Three recent studies have suggested that anoctamin 1 (ANO1), also referred to as TMEM16A, could be involved in apical iodide efflux in thyroid cells (Viitanen et al. 2013; Iosco et al. 2014; Twyffels et al. 2014). ANO1 is a calcium-activated anion channel, which is expressed in numerous tissues, including thyroid follicular cells (Pedemonte and Galietta 2014; Ferrera et al. 2010). ANO1 is part of a family of ten paralogs (ANO1-10; TMEM16A-K) sharing a common transmembrane topology, but a wide spectrum of in part putative functional roles as ion channels, regulatory subunits of other channels or phospholipid scramblases, proteins responsible for the translocation of phospholipids between the two monolayers of the cell membrane lipid bilayer (Pedemonte and Galietta 2014; Picollo et al. 2015). The *ANO1* gene generates several splice variants, and most of them have a higher affinity for iodide than chloride (Ferrera et al. 2010). Human and rat thyroid cells predominantly express the so-called *abc* and the *ac* isoforms (Ferrera et al. 2009; Iosco et al. 2014), whereas the rat thyroid cell lines PCCL3 and FRTL-5 predominantly express the *ac* isoform, which is more sensitive to calcium (Ferrera et al. 2009, 2010). A functional study by Viitanen et al. performed in native FRTL-5 rat thyroid cells suggested that ANO1, in conjunction with the transient receptor potential channel 2 (TRPC2), mediates iodide release (Viitanen et al. 2013). Iosco et al. then demonstrated that the ANO1 protein is localized at the apical membrane of human thyrocytes (Fig. 7.1), and that its expression is more abundant in active cells (Iosco et al. 2014). Functional studies determining the intracellular iodide content revealed that iodide release can be stimulated by adenosine triphosphate (ATP) in a calcium-dependent manner from FRTL-5 cells, whereas treatment with inhibitors or siRNA knockdown decreased the iodide efflux. Similarly, iodide efflux was also increased in transfected mammalian cells expressing both NIS and ANO1, and iodide release could be further stimulated by calcium (Iosco et al. 2014). Twyffels et al. demonstrated that *Ano1* mRNA is stimulated by TSH and that the protein expression, which is relatively discrete under basal conditions, increases after stimulation by TSH (Twyffels et al. 2014). In unpolarized human embryonic kidney (HEK293) cells transfected with ANO1, the efflux of iodide is increased compared to untransfected cells. However, in HEK293 cells expressing pendrin, the iodide efflux is higher compared to ANO1 transfected cells, suggesting that pendrin is more efficient in mediating iodide efflux, at least in this model system (Fig. 7.2). Treatment with the calcium ionophore ionomycin was found to acutely stimulate the ANO1-mediated iodide

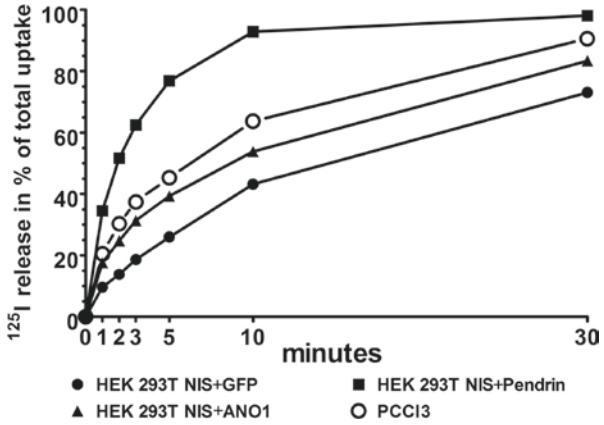

Fig. 7.2 Release of radioiodide from ^{125}I-preloaded PCCl3 cells (○) or HEK 293 T cells transfected to express Na^+/I^- symporter (*NIS*) in combination with *GFP* (●) or *ANO1* (▲) or *pendrin* (■). The data are representative of more than five independent experiments (From Ref. Twyffels et al. (2014). With permission). Note that the release of iodide is more efficient in these pendrin-transfected cells compared to ANO1-transfected cells

release (Twyffels et al. 2014). The mechanism by which calcium activates ANO1 seems to involve calmodulin as well as direct calcium binding to ANO1 (Pedemonte and Galietta 2014). Rat thyroid cell lines and human primary thyroid cells treated either with an ANO1 inhibitor (T16Ainh-A01) or siRNA show a decrease in iodide release (Twyffels et al. 2014). Studies in oocytes have demonstrated that ANO1 is a calcium-activated chloride channel with a preference for iodide over chloride (Schroeder et al. 2008; Yang et al. 2008) indirectly suggesting that it could be involved in mediating iodide efflux. ANO1 is able to mediate iodide efflux from FRTL-5 cells after ATP stimulation in the absence of chloride, which suggests that it functions independently of pendrin, which functions as an anion exchanger. In aggregate, these results indicate that ANO1, which has a preferential affinity for iodide over chloride (Schroeder et al. 2008; Yang et al. 2008), is able to mediate iodide release from thyroid cells.

7.5 Future Directions

In conclusion, the current body of data suggests that the multianion exchanger pendrin (PDS/SLC26A4) and the calcium-dependent channel anoctamin 1 (ANO1/TMEM16A) can mediate apical iodide efflux in thyroid cells and several model systems. It is conceivable that they are part of a redundant system. The exact physiological role of pendrin and ANO1 awaits further characterization, and it may be variable between basal conditions and conditions of thyroid dysfunction as illustrated by the regulation of ANO1 expression by TSH, and the differential regulation of pendrin trafficking by the protein kinase A and C pathways. As suggested by the

absence of a thyroid phenotype in *Slc26a4 −/−* knockout mice, which differs with the human phenotype that can include goiter and congenital or acquired hypothyroidism, there may be relevant differences in the expression pattern and physiological roles of pendrin and ANO1 between species.

References

Alper SL, Sharma AK (2013) The SLC26 gene family of anion transporters and channels. Mol Aspects Med 34(2–3):494–515. doi:10.1016/j.mam.2012.07.009

Azroyan A, Morla L, Crambert G, Laghmani K, Ramakrishnan S, Edwards A, Doucet A (2012) Regulation of pendrin by cAMP: possible involvement in beta-adrenergic-dependent NaCl retention. Am J Physiol Renal Physiol 302(9):F1180–F1187. doi:10.1152/ajprenal.00403.2011

Bizhanova A, Kopp P (2010) Genetics and phenomics of Pendred syndrome. Mol Cell Endocrinol 322(1–2):83–90. doi:10.1016/j.mce.2010.03.006

Bizhanova A, Kopp P (2011) Controversies concerning the role of pendrin as an apical iodide transporter in thyroid follicular cells. Cell Physiol Biochem 28(3):485–490. doi:10.1159/000335103

Bizhanova A, Chew T, Khuon S, Kopp P (2011) Analysis of cellular localization and function of carboxy-terminal truncation mutants of pendrin. Cell Physiol Biochem 28:423–434

Calebiro D, Porazzi P, Bonomi M, Lisi S, Grindati A, De Nittis D, Fugazzola L, Marino M, Botta G, Persani L (2011) Absence of primary hypothyroidism and goiter in *Slc26a4 (−/−)* mice fed on a low iodine diet. J Endocrinol Invest 34:593–598. doi:10.3275/7262, 7262 [pii]

Calil-Silveira J, Serrano-Nascimento C, Kopp P, Nunes MT (2016) Iodide excess regulates its own efflux: a possible involvement of pendrin. Am J Physiol Cell Physiol ajpcell 00210:02015. doi:10.1152/ajpcell.00210.2015

Di Cosmo C, Liao XH, Dumitrescu AM, Philp NJ, Weiss RE, Refetoff S (2010) Mice deficient in MCT8 reveal a mechanism regulating thyroid hormone secretion. J Clin Invest 120(9):3377–3388. doi:10.1172/JCI42113, 42113 [pii]

Di Jeso B, Arvan P (2016) Thyroglobulin from molecular and cellular biology to clinical endocrinology. Endocr Rev 37(1):2–36. doi:10.1210/er.2015-1090

Dossena S, Rodighiero S, Vezzoli V, Nofziger C, Salvioni E, Boccazzi M, Grabmayer E, Botta G, Meyer G, Fugazzola L, Beck-Peccoz P, Paulmichl M (2009) Functional characterization of wild-type and mutated pendrin (SLC26A4), the anion transporter involved in Pendred syndrome. J Mol Endocrinol 43(3):93–103. doi:10.1677/JME-08-0175, JME-08-0175 [pii]

Dossena S, Bizhanova A, Nofziger C, Bernardinelli E, Ramsauer J, Kopp P, Paulmichl M (2011) Identification of allelic variants of pendrin (SLC26A4) with loss and gain of function. Cell Physiol Biochem 28:467–476

Everett LA, Glaser B, Beck JC, Idol JR, Buchs A, Heyman M, Adawi F, Hazani E, Nassir E, Baxevanis AD, Sheffield VC, Green ED (1997) Pendred syndrome is caused by mutations in a putative sulphate transporter gene *(PDS)*. Nat Genet 17(4):411–422. doi:10.1038/ng1297-411

Ferrera L, Caputo A, Ubby I, Bussani E, Zegarra-Moran O, Ravazzolo R, Pagani F, Galietta LJ (2009) Regulation of TMEM16A chloride channel properties by alternative splicing. J Biol Chem 284(48):33360–33368. doi:10.1074/jbc.M109.046607

Ferrera L, Caputo A, Galietta LJ (2010) TMEM16A protein: a new identity for Ca(2+)-dependent Cl(−) channels. Physiology (Bethesda) 25(6):357–363. doi:10.1152/physiol.00030.2010

Frohlich H, Boini KM, Seebohm G, Strutz-Seebohm N, Ureche ON, Foller M, Eichenmuller M, Shumilina E, Pathare G, Singh AK, Seidler U, Pfeifer KE, Lang F (2011) Hypothyroidism

of gene-targeted mice lacking *Kcnq1*. Pflugers Arch 461(1):45–52. doi:10.1007/s00424-010-0890-5

Gillam MP, Sidhaye AR, Lee EJ, Rutishauser J, Stephan CW, Kopp P (2004) Functional characterization of pendrin in a polarized cell system. Evidence for pendrin-mediated apical iodide efflux. J Biol Chem 279(13):13004–13010. doi:10.1074/jbc.M313648200, M313648200 [pii]

Golstein P, Abramow M, Dumont JE, Beauwens R (1992) The iodide channel of the thyroid: a plasma membrane vesicle study. Am J Physiol 263(3 Pt 1):C590–C597

Gonzalez Trevino O, Karamanoglu Arseven O, Ceballos CJ, Vives VI, Ramirez RC, Gomez VV, Medeiros-Neto G, Kopp P (2001) Clinical and molecular analysis of three Mexican families with Pendred's syndrome. Eur J Endocrinol 144(6):585–593, doi:1440585 [pii]

Iosco C, Cosentino C, Sirna L, Romano R, Cursano S, Mongia A, Pompeo G, di Bernardo J, Ceccarelli C, Tallini G, Rhoden KJ (2014) Anoctamin 1 is apically expressed on thyroid follicular cells and contributes to ATP- and calcium-activated iodide efflux. Cell Physiol Biochem 34(3):966–980. doi:10.1159/000366313

Iwata T, Yoshida T, Teranishi M, Murata Y, Hayashi Y, Kanou Y, Griffith AJ, Nakashima T (2011) Influence of dietary iodine deficiency on the thyroid gland in *Slc26a4*-null mutant mice. Thyroid Res 4(1):1–6. doi:10.1186/1756-6614-4-10

Kopp P (2012) Thyroid hormone synthesis: thyroid iodine metabolism. In: Braverman L, Cooper D (eds) Wegner and Ingbar's the thyroid: a fundamental and clinical text, 10th edn. Lippincott, Williams & Wilkins, Philadelphia, pp 48–74

Kopp P (2014) Mutations in the Pendred Syndrome *(PDS/SLC26A)* gene: an increasingly complex phenotypic spectrum from goiter to thyroid hypoplasia. J Clin Endocrinol Metab 99(1):67–69. doi:10.1210/jc.2013-4319

Kühnen P, Turan S, Fröhler S, Güran T, Abali S, Biebermann H, Bereket A, Grüters A, Chen W, Krude H (2014) Identification of PENDRIN *(SLC26A4)* mutations in patients with congenital hypothyroidism and "apparent" thyroid dysgenesis. J Clin Endocrinol Metab 99(1):E169–E176. doi:10.1210/jc.2013-2619

Ladsous M, Vlaeminck-Guillem V, Dumur V, Vincent C, Dubrulle F, Dhaenens CM, Wemeau JL (2014) Analysis of the thyroid phenotype in 42 patients with Pendred syndrome and nonsyndromic enlargement of the vestibular aqueduct. Thyroid 24(4):639–648. doi:10.1089/thy.2013.0164

Li H, Ganta S, Fong P (2010) Altered ion transport by thyroid epithelia from *CFTR(−/−)* pigs suggests mechanisms for hypothyroidism in cystic fibrosis. Exp Physiol 95(12):1132–1144. doi:10.1113/expphysiol.2010.054700

Moreno JC, Visser TJ (2007) New phenotypes in thyroid dyshormonogenesis: hypothyroidism due to *DUOX2* mutations. Endocr Dev 10:99–117. doi:10.1159/0000106822

Moreno JC, Klootwijk W, van Toor H, Pinto G, D'Alessandro M, Leger A, Goudie D, Polak M, Gruters A, Visser TJ (2008) Mutations in the iodotyrosine deiodinase gene and hypothyroidism. N Engl J Med 358(17):1811–1818. doi:10.1056/NEJMoa0706819, 358/17/1811 [pii]

Morgans ME, Trotter WR (1958) Association of congenital deafness with goitre; the nature of the thyroid defect. Lancet 1(7021):607–609

Muscella A, Marsigliante S, Verri T, Urso L, Dimitri C, Botta G, Paulmichl M, Beck-Peccoz P, Fugazzola L, Storelli C (2008) PKC-epsilon-dependent cytosol-to-membrane translocation of pendrin in rat thyroid PC Cl3 cells. J Cell Physiol 217(1):103–112. doi:10.1002/jcp.21478

Nilsson M, Bjorkman U, Ekholm R, Ericson LE (1990) Iodide transport in primary cultured thyroid follicle cells: evidence of a TSH-regulated channel mediating iodide efflux selectively across the apical domain of the plasma membrane. Eur J Cell Biol 52(2):270–281

Nilsson M, Bjorkman U, Ekholm R, Ericson LE (1992) Polarized efflux of iodide in porcine thyrocytes occurs via a cAMP-regulated iodide channel in the apical plasma membrane. Acta Endocrinol 126(1):67–74

Paroder V, Spencer SR, Paroder M, Arango D, Schwartz S Jr, Mariadason JM, Augenlicht LH, Eskandari S, Carrasco N (2006) Na(+)/monocarboxylate transport (SMCT) protein expression correlates with survival in colon cancer: molecular characterization of SMCT. Proc Natl Acad Sci U S A 103(19):7270–7275. doi:10.1073/pnas.0602365103, 0602365103 [pii]

Pedemonte N, Galietta LJ (2014) Structure and function of TMEM16 proteins (anoctamins). Physiol Rev 94(2):419–459. doi:10.1152/physrev.00039.2011

Pendred V (1896) Deaf-mutism and goitre. Lancet ii:532

Pera A, Dossena S, Rodighiero S, Gandia M, Botta G, Meyer G, Moreno F, Nofziger C, Hernandez-Chico C, Paulmichl M (2008) Functional assessment of allelic variants in the *SLC26A4* gene involved in Pendred syndrome and nonsyndromic EVA. Proc Natl Acad Sci U S A 105(47):18608–18613. doi:10.1073/pnas.0805831105, 0805831105 [pii]

Pesce L, Kopp P (2014) Iodide transport: implications for health and disease. Int J Pediatr Endocrinol 8:1–12. doi:10.1186/1687-9856-2014-8

Pesce L, Bizhanova A, Caraballo JC, Westphal W, Butti ML, Comellas A, Kopp P (2012) TSH regulates pendrin membrane abundance and enhances iodide efflux in thyroid cells. Endocrinology 153(1):512–521. doi:10.1210/en.2011-1548

Picollo A, Malvezzi M, Accardi A (2015) TMEM16 proteins: unknown structure and confusing functions. J Mol Biol 427(1):94–105. doi:10.1016/j.jmb.2014.09.028

Portulano C, Paroder-Belenitsky M, Carrasco N (2014) The Na+/I- symporter (NIS): mechanism and medical impact. Endocr Rev 35(1):106–149. doi:10.1210/er.2012-1036

Rodriguez AM, Perron B, Lacroix L, Caillou B, Leblanc G, Schlumberger M, Bidart JM, Pourcher T (2002) Identification and characterization of a putative human iodide transporter located at the apical membrane of thyrocytes. J Clin Endocrinol Metab 87(7):3500–3503

Roepke TK, King EC, Reyna-Neyra A, Paroder M, Purtell K, Koba W, Fine E, Lerner DJ, Carrasco N, Abbott GW (2009) *Kcne2* deletion uncovers its crucial role in thyroid hormone biosynthesis. Nat Med 15(10):1186–1194. doi:10.1038/nm.2029

Rotman-Pikielny P, Hirschberg K, Maruvada P, Suzuki K, Royaux IE, Green ED, Kohn LD, Lippincott-Schwartz J, Yen PM (2002) Retention of pendrin in the endoplasmic reticulum is a major mechanism for Pendred syndrome. Hum Mol Genet 11(21):2625–2633

Royaux IE, Suzuki K, Mori A, Katoh R, Everett LA, Kohn LD, Green ED (2000) Pendrin, the protein encoded by the Pendred syndrome gene *(PDS)*, is an apical porter of iodide in the thyroid and is regulated by thyroglobulin in FRTL-5 cells. Endocrinology 141(2):839–845

Sato E, Nakashima T, Miura Y, Furuhashi A, Nakayama A, Mori N, Murakami H, Naganawa S, Tadokoro M (2001) Phenotypes associated with replacement of His by Arg in the Pendred syndrome gene. Eur J Endocrinol 145(6):697–703, doi:145697 [pii]

Schroeder BC, Cheng T, Jan YN, Jan LY (2008) Expression cloning of TMEM16A as a calcium-activated chloride channel subunit. Cell 134(6):1019–1029. doi:10.1016/j.cell.2008.09.003

Scott DA, Wang R, Kreman TM, Sheffield VC, Karniski LP (1999) The Pendred syndrome gene encodes a chloride-iodide transport protein. Nat Genet 21(4):440–443. doi:10.1038/7783

Shcheynikov N, Yang D, Wang Y, Zeng W, Karniski LP, So I, Wall SM, Muallem S (2008) The Slc26a4 transporter functions as an electroneutral Cl-/I-/HCO3- exchanger: role of Slc26a4 and Slc26a6 in I- and HCO3- secretion and in regulation of CFTR in the parotid duct. J Physiol 586(16):3813–3824. doi:10.1113/jphysiol.2008.154468

Silveira JC, Kopp PA (2015) Pendrin and anoctamin as mediators of apical iodide efflux in thyroid cells. Curr Opin Endocrinol Diabetes Obes 22(5):374–380. doi:10.1097/MED.0000000000000188

Taylor JP, Metcalfe RA, Watson PF, Weetman AP, Trembath RC (2002) Mutations of the PDS gene, encoding pendrin, are associated with protein mislocalization and loss of iodide efflux: implications for thyroid dysfunction in Pendred syndrome. J Clin Endocrinol Metab 87(4):1778–1784

Twyffels L, Massart C, Golstein PE, Raspe E, Van Sande J, Dumont JE, Beauwens R, Kruys V (2011) Pendrin: the thyrocyte apical membrane iodide transporter? Cell Physiol Biochem 28(3):491–496. doi:10.1159/000335110

Twyffels L, Strickaert A, Virreira M, Massart C, Van Sande J, Wauquier C, Beauwens R, Dumont JE, Galietta LJ, Boom A, Kruys V (2014) Anoctamin-1/TMEM16A is the major apical iodide channel of the thyrocyte. Am J Physiol Cell Physiol 307(12):C1102–C1112. doi:10.1152/ajpcell.00126.2014

van den Hove MF, Croizet-Berger K, Jouret F, Guggino SE, Guggino WB, Devuyst O, Courtoy PJ (2006) The loss of the chloride channel, ClC-5, delays apical iodide efflux and induces a

euthyroid goiter in the mouse thyroid gland. Endocrinology 147(3):1287–1296. doi:10.1210/en.2005-1149, en.2005-1149 [pii]

Viitanen TM, Sukumaran P, Lof C, Tornquist K (2013) Functional coupling of TRPC2 cation channels and the calcium-activated anion channels in rat thyroid cells: implications for iodide homeostasis. J Cell Physiol 228(4):814–823. doi:10.1002/jcp.24230

Weiss SJ, Philp NJ, Grollman EF (1984) Effect of thyrotropin on iodide efflux in FRTL-5 cells mediated by Ca2+. Endocrinology 114(4):1108–1113

Yang YD, Cho H, Koo JY, Tak MH, Cho Y, Shim WS, Park SP, Lee J, Lee B, Kim BM, Raouf R, Shin YK, Oh U (2008) TMEM16A confers receptor-activated calcium-dependent chloride conductance. Nature 455(7217):1210–1215. doi:10.1038/nature07313

Yoshida A, Taniguchi S, Hisatome I, Royaux IE, Green ED, Kohn LD, Suzuki K (2002) Pendrin is an iodide-specific apical porter responsible for iodide efflux from thyroid cells. J Clin Endocrinol Metab 87(7):3356–3361