The role of pendrin in blood pressure regulation

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Pendrin is a Na+/HCO3− exchanger found in the apical regions of type B and non-A, non-B intercalated cells within the aldosterone-sensitive region of the nephron, i.e., the distal convoluted tubule (DCT), the connecting tubule (CNT), and the cortical collecting duct (CCD). Type B intercalated cells mediate Cl− absorption and HCO3− secretion primarily through pendrin-mediated Cl−/HCO3− exchange. This exchanger is upregulated with angiotensin II administration and in models of metabolic alkalosis, such as following administration of aldosterone or NaHCO3. In the absence of pendrin-mediated HCO3− secretion, an enhanced alkalosis is observed following aldosterone or NaHCO3 administration. However, probably of more significance is the role of pendrin in the pressor response to aldosterone. Pendrin mediates Cl− absorption and modulates aldosterone-induced Na+ absorption mediated by the epithelial Na channel (ENaC). Pendrin changes ENaC activity by changing both channel open probability (Po) and surface density (N), at least partly by altering luminal HCO3− and ATP concentration. Thus aldosterone and angiotensin II stimulate pendrin expression and function, which stimulates ENaC activity, thereby contributing to the pressor response of these hormones. However, pendrin may modulate blood pressure partly through its extrarenal effects. For example, pendrin is expressed in the adrenal medulla, where it modulates catecholamine release. The increase in catecholamine release observed with pendrin gene ablation likely contributes to the increment in vascular contractile force observed in the pendrin null mouse. This review summarizes the signaling mechanisms that regulate pendrin abundance and function as well as the contribution of pendrin to distal nephron function.

Slc26a4; Pds; Cl−/HCO3− exchange; ENaC; pendrin; intercalated cells; blood pressure regulation.

The kidney helps restore fluid balance following volume contraction in part by increasing distal nephron NaCl absorption. Conversely, during volume overload the kidney reduces distal nephron NaCl absorption. Most distal NaCl transport occurs within the distal convoluted tubule (DCT), the connecting tubule (CNT), and the cortical collecting duct (CCD) (88). The medullary collecting duct mediates less net NaCl absorption than these more proximal segments, but is nevertheless important in that it performs the final regulation of NaCl balance (88).

Ion transport in the CCD and CNT has been characterized using CCDs and CNTs perfused in vitro. However, many more studies have been done in the CCD than the CNT because of the technical challenges associated with perfusing CNTs in vitro. These studies demonstrated that Na+ and probably Cl− absorption in the CCD is less than half of that found in the CNT of untreated rabbits (2). Moreover, because total tubule length in vivo is sixfold greater in the CNT than in the CCD (88), NaCl absorption is at least an order of magnitude greater in the CNT than that in CCD in vivo. The two cell types found within the CCD are principal and intercalated cells, whereas the CNT harbors CNT cells (2) and intercalated cells. In this review, we will refer to both CNT cells and principal cells as principal cells. Na+ is absorbed primarily by principal cells, whereas Cl− absorption occurs predominantly across intercalated cells or possibly through paracellular transport (64). Intercalated cells are classified as type A, type B or non-A, non-B cells, based on the expression of the Cl−/HCO3− exchanger, AE1, and the subcellular distribution of the H+−ATPase within the cell (3, 9, 30, 77) (Fig. 1), which predicts whether the cell will secrete H+ or OH− equivalents.

Pendrin is an electroneutral Na+−independent, Cl−/HCO3− exchanger that is expressed in both the CNT and the CCD in the apical regions of type B and non-A, non-B intercalated cells, where it plays a critical role in the Cl− absorption and HCO3− secretion mediated by these cell types (31, 63, 86). Because total and apical plasma membrane pendrin protein abundance is higher in the CNT than the CCD under both basal and stimulated conditions (82, 86) and because of the greater length of the CNT than the CCD, pendrin probably modulates ion transport more along the CNT than the CCD. As will be described in this review, while pendrin-mediated HCO3− secretion plays a role in the renal regulation of acid-base balance, pendrin-mediated Cl− absorption is probably the more physiologically significant. Pendrin regulates vascular volume and blood pressure by mediating renal Cl− absorption. Moreover,
In 1896, Dr. Vaughan Pendred (56) reported a family in which 2 of the 10 children suffered from congenital deafness and goiter. The syndrome that bears his name (Pendred syndrome) is inherited in an autosomal recessive fashion and is seen in 7.5 per 100,000 persons (16). In 1997, Everett and colleagues (16) identified the gene responsible for this syndrome by positional cloning. This gene is now called Slc26a4, and the protein it encodes is called pendrin. Determining the Slc26a4 gene sequence enabled better study of pendrin-mediated transport and better study of pendrin’s distribution and its regulation.

Cloning the Gene Responsible for Pendred Syndrome

High levels of pendrin expression have been detected in the thyroid and inner ear (62, 89), as expected given the ear and thyroid phenotype observed in Pendred syndrome. However, surprisingly high levels of pendrin protein expression were also observed in the kidney (63). In particular, pendrin expression was found in type B and non-A, non-B intercalated cells, which are a minority cell type found within the aldosterone-sensitive region of the nephron, i.e., the distal portion of DCT, the CNT, and the CCD (31, 63, 86).

When expressed in heterologous expression systems, pendrin acts as an electroneutral, Na+ independent, Cl−/HCO3− and Cl−/Cl− and Cl−/I− exchanger (67–69, 72). Because pendrin localizes to the apical regions of cells known to secrete HCO3− and absorb Cl− through Na+ independent, electroneutral exchange (63), we hypothesized that pendrin mediates the Cl− absorption and HCO3− secretion observed in the rodent CCD. To test this hypothesis, we examined the effect of pendrin gene ablation on Cl− and total CO2 (HCO3−) transport in mouse CCDs perfused in vitro (63, 87). Mice were given an aldosterone analog and NaHCO3 in their drinking water to upregulate apical Cl−/HCO3− exchange in the type B cell (34, 73). We observed HCO3− secretion and Cl− absorption in wild-type CCDs, but not in CCDs from pendrin null mice. Therefore, type B intercalated cell apical Cl−/HCO3− exchange is pendrin-dependent.

Within the CCD, Cl− absorption occurs through two distinct mechanisms, which differ in their sensitivity to different di-
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The first is sensitive to ENaC inhibitors, such as amiloride, whereas the second is sensitive to thiazides, which are well known to inhibit the NaCl cotransporter (NCC; encoded by Slc12a3) (78). The relative contribution of the thiazide- and the amiloride-sensitive components of NaCl absorption to total NaCl absorption varies between studies and probably depends on the treatment model employed (37, 78). While thiazides strongly inhibit NCC in the DCT, NCC cannot mediate the thiazide-sensitive Cl− absorption in the CCD since NCC is not expressed in that segment (37). Leviel and collaborators (37) showed, however, that thiazide-sensitive Cl− absorption in the CCD occurs through the Na+/HCO3− exchange (NDCBE) encoded by Slc4a8. During dietary NaCl restriction, electroneutral NaCl absorption in the CCD occurs through pendrin-mediated Cl−/HCO3− exchange acting in tandem with NDCBE-mediated Na+/HCO3− exchange. While NDCBE stoichiometry has not been established, it is thought to mediate secretion of Cl− and the absorption of Na+ and HCO3− (Fig. 2) through electrogenic exchange. In contrast, pendrin mediates electroneutral Cl− absorption and HCO3− secretion. Therefore, with three pendrin and one NDCBE molecule acting in tandem, some recycling of Cl− and HCO3− across the apical membrane is expected although, net NaCl absorption and net HCO3− secretion are predicted (Fig. 2) (37). However, if one pendrin and two NDCBE molecules act in tandem, net Cl− secretion and HCO3− absorption are predicted (Fig. 2). Although functional data have shown that this Na+/HCO3− exchanger localizes to intercalated cells (37), the protein localization of NDCBE to intercalated cells, however, has not been confirmed.

ENaC inhibitors reduce Cl− absorption in the CCD, although ENaC does not directly mediate Cl− transport. As such, ENaC blockade must alter Cl− exchange or channel activity either by changing the release of a signaling molecule that regulates this transporter/channel or by modulating the driving force for Cl− movement mediated by another Cl− transport mechanism. While the Cl− transport mechanism targeted by ENaC inhibition is unknown, it does not appear to be mediated by pendrin, CIC-5, or CFTR (46, 51, 52). Amiloride-sensitive Cl− absorption may occur instead through paracellular Cl− transport or through transcellular Cl− transport mediated by an electrogenic Cl− exchanger or a Cl− channel. For example, the lumen-negative V0 generated by ENaC should provide the driving force for paracellular Cl− absorption across tight junctions. Claudin 4 localizes to tight junctions between principal and intercalated cells and acts as a Cl− channel (20, 26). Following claudin 4 gene ablation, a natriuresis, a chloriuresis, and reduced blood pressure are observed, particularly following dietary NaCl restriction (20). As such, ENaC blockade in the CCD may reduce electrogenic Cl− absorption by eliminating the driving force for claudin 4-mediated Cl− transport across tight junctions.

Pendrin is Upregulated with Aldosterone

Since pendrin colocalizes with the putative apical anion exchanger of the type B intercalated cell, we asked whether the regulation of pendrin and apical anion exchange activity is similar. Since aldosterone administration increases Cl− absorption in the CCD by stimulating apical Na+/HCO3− independent, electroneutral Cl−/HCO3− exchange (73), further studies explored the effect of aldosterone on renal pendrin abundance and subcellular distribution. We observed that while B cell apical plasma membrane immunoreactivity is low under basal conditions (82, 86), type B intercalated cell apical plasma membrane pendrin abundance increases sixfold in response to aldosterone, primarily through subcellular redistribution (82). In the absence of pendrin (pendrin knockout mice), the aldosterone-induced increment in Cl− absorption and HCO3− secretion observed in the CCD of wild-type mice (18, 25, 34, 73) is greatly reduced (63, 87). Therefore, type B intercalated cell apical Cl−/HCO3− exchange is primarily pendrin mediated.

Because pendrin mediates HCO3− secretion, further studies examined the effect of pendrin gene ablation on acid-base balance. As expected, we observed that pendrin gene ablation enhanced the metabolic alkalosis observed with the administration of aldosterone analogs. However, these studies also
produced the unanticipated finding that pendrin is important in blood pressure regulation (82).

Pendrin Regulates Blood Pressure

Since pendrin transports Cl⁻, we explored its role in NaCl balance (87). Following a NaCl-replete diet, apparent vascular volume is similar in wild-type and in pendrin null mice (84, 87). However, following dietary NaCl restriction, where renin and aldosterone release are appropriately stimulated, pendrin null mice excrete more NaCl, lose more body weight, have lower blood pressure and have a greater increase in blood urea nitrogen concentration relative to wild-type mice, consistent with greater apparent vascular volume contraction in the mutant than in the wild-type mice (32, 50, 84). Therefore, the lower blood pressure observed in the mutant mice is due at least in part to the impaired ability of the pendrin null kidney to fully conserve NaCl (32, 84, 87). Differences in blood pressure between pendrin null and wild-type mice are greater in treatment models that upregulate pendrin, such as during dietary NaCl restriction or aldosterone infusion (50, 82, 84, 87). The marked impact of intercalated cell Cl⁻ transporters on blood pressure and vascular volume regulation was surprising since intercalated cells make up only ~1% of total kidney volume (30, 88).

Whereas pendrin null are hypertensive, mice that overexpress pendrin have salt-sensitive hypertension (28). In particular, blood pressure is very sensitive to dietary intake of Cl⁻ in mice overexpressing pendrin (28). As such, whether the NaCl-sensitive hypertension observed in people occurs in part from increased pendrin-mediated Cl⁻ absorption has received a great deal of interest. While the physiological role of pendrin in the pathogenesis of hypertension has not been explored fully, the incidence of hypertension was compared in a retrospective study was inconclusive, it raised the possibility that pendrin gene ablation is protective against the development of hypertension.

Pendrin Modulates the Aldosterone-Induced Increment in ENaC Abundance and Function

In the absence of pendrin-mediated Cl⁻ absorption, a chlor riuresis and a natriuresis are observed in pendrin null mice following dietary NaCl restriction. Both the natriuresis as well as the chlor riuresis observed with pendrin gene ablation should affect blood pressure (32). However, because pendrin does not directly mediate Na⁺ transport, we asked whether pendrin modulates the expression of a major renal Na⁺ transporter. To answer this question, renal Na⁺ transporter abundance was quantified in kidneys from wild-type and pendrin null mice following treatment models in which the renin-angiotensin-aldosterone axis was either stimulated or suppressed (32). Pendrin gene ablation did not significantly alter renal Na⁺ transporter abundance when mice consumed a NaCl-replete diet, which suppresses circulating plasma renin and aldosterone concentration. In particular, Na⁺/H⁺ exchanger isofor 3 (NHE3), α1-Na-K-ATPase, Na⁺-K⁺-2Cl⁻ cotransporter (NKCC2), ENaC, and NCC abundance was similar in lysates from wild-type and Slc26a4 null mice following a balanced, NaCl-replete diet (32). However, following dietary NaCl restriction or with an aldosterone infusion, pendrin gene ablation reduces α-, β- and γ-ENaC subunit abundance in the kidney (32, 50, 53). In particular, pendrin gene ablation blunts the aldosterone-induced increment in 70-kDa γ-ENaC abundance. Therefore, pendrin modulates ENaC subunit abundance.

Since pendrin gene ablation reduces ENaC subunit abundance, we explored ENaC function in wild-type and pendrin null mice. Administration of aldosterone in vivo markedly stimulates Na⁺ absorption in the rat and mouse CCD, which is nearly eliminated with the application of an ENaC inhibitor (53, 74). However, the aldosterone-induced increment in Na⁺ absorption observed in the CCD is dependent on pendrin expression. In CCDs from aldosterone-treated mice, pendrin gene ablation reduces Na⁺ absorption by ~60% (53). To further explore the effect of pendrin gene ablation on ENaC function, we measured transepithelial voltage, Vₜ, in CCDs perfused in vitro and after the application of benzamil to the luminal fluid (32). The change in voltage observed with benzamil application was taken as an index of ENaC-mediated Na⁺ absorption. In CCDs from aldosterone-treated wild-type mice, we observed a substantial lumen-negative Vₜ, which was obliterated with benzamil application. However, the lumen-negative Vₜ as well as the change in Vₜ observed with the application of benzamil was much lower in CCDs from aldo sterone-treated pendrin null mice (32, 53). We conclude that pendrin gene ablation markedly reduces ENaC-mediated Na⁺ absorption in CCDs from aldosterone-treated mice. This reduction in ENaC function contributes to the lower blood pressure observed in these mutant mice.

Aldosterone increases ENaC activity, NＰα, through three mechanisms. First, it increases the frequency at which the channel is open (open probability, Pₒ) by postranslational modification involving subunit cleavage (19). Second, it increases ENaC subunit protein abundance, particularly that of the α-subunit. Finally, it increases the abundance of ENaC subunits that localize to the plasma membrane relative to the total cellular subunit pool (41). Aldosterone induces a redistribution of subunits from the intracellular pool to the apical plasma membrane. For aldosterone to efficiently deliver ENaC to the apical plasma membrane, assembly of all three ENaC subunits is required (27, 45, 80). As will be discussed below, aldosterone increases channel residency time in the apical plasma membrane, in part, through Sgk1 phosphorylation, which prevents the ubiquitin ligase, NEDD4-2, from associating with and ubiquitinating ENaC. This increase in ENaC subunit total protein abundance and the increased subunit abundance in the region of the apical plasma membrane (17) contribute to the increase in membrane channel density observed with aldosterone application (4).

Further experiments examined the effect of pendrin gene ablation on channel activity, NＰα, channel surface density, N, and channel open probability, Pₒ, in principal cells of split open mouse CCDs taken from aldosterone-treated mice (53). We observed that pendrin gene ablation reduced channel surface density, N, by ~50%, which is consistent with the fall in ENaC subunit total protein abundance as well as the reduced subunit immunolabel in the region of the apical plasma membrane relative to total label in the same cell (32, 50, 53). Therefore, pendrin gene ablation reduces principal cell channel density due to a decline in total subunit protein abundance and from subcellular redistribution, which reduces relative subunit abundance in the region of the apical membrane.
Other experiments explored whether pendrin gene ablation also attenuates ENaC activity by reducing channel $P_o$ (53). Following aldosterone administration, the $\alpha$- and $\gamma$-ENaC subunits undergo two cleavage events, which raise channel activity by increasing channel $P_o$ (10, 11, 44). We asked whether ENaC activity is lower in principal cells from aldosterone-treated pendrin null relative to wild-type mice due to an impairment in these cleavage events. To test this hypothesis, we examined ENaC activity after the application of a subunit cleaving protease (trypsin) (53). To do so, we measured $V_T$ in CCDs perfused in vitro from aldosterone-treated wild-type and pendrin null mice before and after the application of trypsin to the perfusate. Trypsin did not change $V_T$ in CCDs from aldosterone-treated wild-type mice, suggesting that the $\alpha$- and $\gamma$-ENaC subunits are fully cleaved. However, in CCDs from aldosterone-treated pendrin null mice, trypsin application increased the luminal-negative $V_T$ by $\sim$3 mV. One interpretation of these observations is that pendrin gene ablation lowers ENaC channel $P_o$ by blunting subunit processing, such as $\alpha$- and/or $\gamma$-subunit cleavage. In other experiments we therefore explored the effect of pendrin gene ablation on channel $P_o$ (53). We observed that in in split-open CCDs from aldosterone-treated mice, pendrin gene ablation reduced channel $P_o$ by $\sim$50%.

In Liddle’s syndrome, ENaC is constitutively upregulated due to a truncation mutation in a region of the $\beta$- or $\gamma$-subunit, known as the PY motif. Elimination of the PY motif prevents the association of NEDD4-2 with ENaC, thereby impairing channel endocytosis and degradation, which increases apical plasma membrane ENaC subunit abundance (14, 53, 60). However, the Liddle’s mutation also increases channel $P_o$ (14, 53, 60). Consistent with these previous studies, we also observed increased ENaC activity in mouse models of Liddle’s syndrome, due in part to increased ENaC subunit abundance in the region of the apical membrane.

Because pendrin reduces ENaC subunit abundance as well as the relative subunit abundance in the region of the apical membrane, we hypothesized that pendrin gene ablation will reduce membrane channel density as well as total and apical membrane subunit abundance less in mice harboring the Liddle’s mutation than in mice with wild-type ENaC, due to the impaired subunit endocytosis and degradation observed in Liddle’s syndrome (53). Therefore, we examined the effect of pendrin gene ablation on channel surface density, $N$, subunit total protein abundance, or subcellular distribution in mouse models of Liddle’s syndrome (53). We observed that pendrin gene ablation changed channel surface density, subunit total protein abundance, and subunit subcellular distribution less in Liddle’s mice than in mice harboring wild-type ENaC. Since Liddle’s mutation blunts the effect of pendrin gene ablation on channel surface density, $N$, we further hypothesized that pendrin gene ablation will reduce ENaC activity less in Liddle’s mice than in mice harboring wild-type ENaC. However, we observed instead that pendrin gene ablation reduced ENaC activity the same or more in CCDs from Liddle’s mice than in mice with wild-type ENaC (53). In Liddle’s mice, pendrin gene ablation reduced channel activity almost exclusively through reduced channel $P_o$ (53). Therefore, in aldosterone-treated mouse models of Liddle’s syndrome, pendrin gene ablation reduced channel activity primarily through changes in channel $P_o$.

**Pendrin Modulates ENaC Abundance in the Kidney by Changing Downstream Luminal Fluid Composition**

Further studies by us and others explored the mechanism by which pendrin changes ENaC abundance and function (22, 50). In human and rodent kidneys, pendrin and ENaC both localize to the aldosterone-sensitive region of the nephron (31, 63, 86). Because ENaC and pendrin localize to different cell types (24, 31, 63, 86) (Fig. 1), there can be no direct ENaC/pendrin protein-protein interaction. Moreover, pendrin does not modulate ENaC by changing the circulating level of a hormone that regulates ENaC, such as vasopressin, aldosterone, angiotensin II, corticosterone, or thyroid hormone (32). Finally, pendrin gene ablation appears to reduce ENaC abundance and function only in the kidney, since ENaC abundance is unchanged in the thyroid or colon of pendrin null mice (32) and since ENaC expression and function are paradoxically increased in the inner ear of these mutant mice (29).

Because ENaC may be pH sensitive (50), we hypothesized that pendrin-mediated $\text{Cl}^-/\text{HCO}_3^-$ exchange raises luminal $\text{HCO}_3^-$ concentration, thereby stimulating ENaC. To test this hypothesis, we gave mice NaHCO$_3$ and aldosterone to stimulate pendrin-mediated HCO$_3^-$ secretion in the CDD (34, 50), which should increase HCO$_3^-$ concentration in the luminal fluid of the CNT and CDD in vivo. We also employed carbonic anhydrase inhibitors to stimulate distal delivery of HCO$_3^-$ independently of pendrin, since carbonic anhydrase inhibitors increase distal HCO$_3^-$ delivery from upstream segments, while greatly downregulating pendrin expression and pendrin-mediated ion transport (43, 50). Therefore, wild-type and pendrin null mice were given aldosterone and NaHCO$_3$ or aldosterone and NaHCO$_3$ plus a carbonic anhydrase inhibitor (acetazolamide). When treated with aldosterone and NaHCO$_3$, we observed that pendrin null mice developed a more severe metabolic alkalosis and had lower renal ENaC subunit abundance and function relative to wild-type (50). However, when mice received this treatment plus acetazolamide, acid-base balance as well as ENaC subunit abundance and function were similar in kidneys from wild-type and pendrin null mice (50). Therefore, stimulating distal HCO$_3^-$ delivery from upstream segments rescues pendrin null mice from the expected fall in renal ENaC abundance and function.

Further studies used cultured mouse principal cells (mpkCCD) to determine whether HCO$_3^-$ concentration directly modulates ENaC abundance and function (50). We observed that when HCO$_3^-$ concentration on the apical side of the monolayer is increased, ENaC abundance and function rise, independently of substituting anions. Therefore, pendrin modulates ENaC, at least in part, by raising luminal HCO$_3^-$ concentration. It remains to be determined whether luminal HCO$_3^-$ concentration directly regulates ENaC or whether HCO$_3^-$ regulates ENaC by changing luminal pH.

While our studies demonstrated that increased extracellular pH or HCO$_3^-$ concentration stimulate ENaC abundance and function (50), other groups have observed an opposite effect (13). In heterologous expression systems, Snyder and colleagues (13) observed that channel activity rises when extracellular pH is reduced. The reason for the discrepancy between
these studies and observations in our laboratory is unclear but may result from the difference in experimental time course. Whereas the Snyder group studied ENaC activity when extracellular pH was varied over <1 min (13), we examined changes in ENaC activity and abundance when extracellular pH was varied over at least 3 h (50). However, these differences may result from other factors, such as differences between native and cultured cells or species-specific differences (13, 50).

Since pendrin gene ablation markedly downregulates H⁺-ATPase abundance in type B intercalated cells (33), pendrin gene ablation may reduce ENaC function due to the expected fall in B cell H⁺-ATPase function. Following H⁺-ATPase gene ablation, Gueutin et al. (22) observed reduced renal cortical ENaC abundance and function. Because B1-H⁺-ATPase null mice have markedly increased urinary excretion of PGE₂, they hypothesized that B cell H⁺-ATPase null mice have markedly increased urinary excretion of PGE₂, they hypothesized that B cell H⁺-ATPase gene ablation downregulates ENaC through a PGE₂-mediated mechanism. They observed that following B cell H⁺-ATPase blockade, intracellular calcium increases dramatically which increases PGE₂ release. This PGE₂ release is blocked with either purinergic receptor blockers or ATP scavengers. Because ATP is secreted into the luminal fluid through connexin 30 hemichannels that localize to the apical plasma membrane of intercalated cells (42), they concluded intercalated cells modulate ENaC through luminal ATP-mediated signaling. When the B cell H⁺-ATPase is inhibited, such as through pendrin gene ablation, connexin-30-mediated ATP secretion increases, which raises luminal ATP concentration (22) (Fig. 3). Luminal ATP acts through apical purinergic receptors to stimulate Ca²⁺ release within principal cells, which increases PGE₂ production, thereby attenuating ENaC abundance and function (22) (Fig. 3). These studies are consistent with an extensive body of work showing that luminal ATP acts through purinergic receptors on the principal cell apical plasma membrane to reduce phosphatidylinositol 4,5-bisphosphate (PIP₂) through a PLC-dependent pathway, thereby lowering ENaC subunit abundance and function (38, 59, 90).

In summary, recent data indicate that pendrin gene ablation reduces ENaC abundance and function by changing the downstream luminal concentration of ATP and HCO₃⁻. However, other pathways may contribute to this interaction between principal and intercalated cells.

**Pendrin and NCC May Compensate for Loss of the Other**

Because pendrin regulates acid-base and fluid and electrolyte balance in the mouse, there has been great interest as to effect of pendrin gene ablation on acid-base and fluid and electrolyte balance in people. While Slc26a4/SLC26A4 expression is high in both the rodent and human kidney, no acid-base or fluid and electrolyte abnormalities are seen under basal conditions in people with Pendred syndrome (55), or in mouse models of Pendred syndrome (82, 87). However, pendrin gene ablation changes acid-base and fluid and electrolyte balance under treatment conditions that stimulate pendrin expression, such as with aldosterone or NaHCO₃ administration, presumably from the reduced capacity of the mutant mice to secrete HCO₃⁻ and absorb Cl⁻ (82, 84, 87).

The lack of an observed renal phenotype in pendrin null mice under basal conditions may occur from compensatory changes by other renal Cl⁻ and/or H⁺/OH⁻ transporters. For example, ablation of the gene encoding NCC (encoded by Slc12A3) upregulates renal pendrin and NDCBE protein abundance (81). This increase in pendrin-NDCBE abundance may compensate for the absence of NCC-mediated NaCl absorption and may explain the lack of significant changes in blood pressure or NaCl balance in NCC null mice given a NaCl-replete diet (basal conditions) (65). Conversely, while pendrin knockout mice do not have significant acid-base abnormalities under basal conditions, with ablation of the genes encoding both pendrin (Slc26a4) and

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*Fig. 3. Pendrin gene ablation changes ENaC abundance and function by modulating luminal ATP concentration. Pendrin gene ablation markedly reduces H⁺-ATPase abundance in the type B intercalated cell. With reduced type B cell H⁺-ATPase expression, ATP content rises, thereby stimulating ATP secretion into the luminal fluid through connexin 30. Luminal ATP acts through apical membrane purinergic receptors to stimulate calcium release, which augments the production of PGE₂. PGE₂ acts through a receptor-mediated mechanism to reduce ENaC abundance and function. Modified with permission from the Annual Review of Physiology, Volume 77 © 2015 by Annual Reviews, http://www.annualreviews.org*
NCC (Slc12A3), profound metabolic alkalosis and vascular volume contraction are observed (71). Moreover, in people with Pendred syndrome, blocking the NaCl cotransporter with thiazides has led to profound metabolic alkalosis and apparent vascular volume contraction (55).

How NCC and pendrin interact to maintain acid-base and fluid and electrolyte balance has been the subject of intense interest. Pendrin abundance and function are markedly increased not only with NCC gene ablation (37, 71, 81) but also with elimination of the NCC activation that follows NCC phosphorylation by STE20/SPS-10-related proline-alanine-rich protein kinase (SPAK) (21). While NCC-mediated NaCl absorption should be low in the absence of SPAK, i.e., in SPAK null mice, neither NCC nor SPAK null mice develop significant vascular volume contraction or hypotension, presumably due to compensation from other renal NaCl transport pathways, such as pendrin (21). While pendrin is upregulated following either either NCC gene ablation or elimination of NCC activation by SPAK gene ablation, chemical inhibitors of NCC, such as hydrochlorothiazide, reduce pendrin abundance, at least under some treatment conditions. Vallet et al. (81) observed that renal pendrin total protein abundance falls after 2 days of thiazide administration at a high dose (130 mg·kg body wt \(^{-1}·\text{day}^{-1}\)) (81). It is possible that hydrochlorothiazide stimulates pendrin abundance and function when given at low doses, wherein the drug selectively inhibits NCC, but inhibits pendrin at higher doses due to carbonic anhydrase inhibition (76).

How pendrin is stimulated following elimination of NCC activation by SPAK gene ablation is not fully resolved, but is mediated at least partly through \(\alpha\)-ketoglutarate (\(\alpha\)-KG) signaling (21). SPAK gene ablation stimulates \(\alpha\)-KG production by the proximal tubule, which is then secreted into the luminal fluid. \(\alpha\)-KG is carried downstream to more distal regions of the nephron (21), where it acts on the O2x1 receptor on the apical plasma membrane of the type B intercalated cell to stimulate pendrin abundance and function (79). How SPAK gene ablation stimulates \(\alpha\)-KG synthesis and/or release remains to be determined, but it may be from the hypokalemia and/or the increased release of angiotensin II observed with SPAK gene ablation (21).

**Pendrin and ENaC are Upregulated by Angiotensin II**

Angiotensin II increases blood pressure in part by stimulating ENaC-mediated renal Na\(^{+}\) absorption in the CNT and collecting duct through both short- and long-term effects (7, 8, 57). Our laboratory asked whether angiotensin II also increases Cl\(^{-}\) absorption in the CCD and explored the mechanism by which it occurs (49). Mice were given furosemide in their food to increase renal pendrin protein abundance (49). We observed that angiotensin II application in vitro increases Cl\(^{-}\) absorption twofold in CNTs and CCDs from furosemide-treated wild-type, but not from pendrin null mice (49). Moreover, since angiotensin II changed Cl\(^{-}\) flux without changing \(V_T\) (49), this peptide hormone must increase Cl\(^{-}\) absorption through a transcellular rather than a paracellular transport mechanism (49).

We reasoned that angiotensin II might increase Cl\(^{-}\) absorption in the CCD by increasing apical plasma membrane pendrin abundance. Alternatively, since the basolateral plasma membrane H\(^{+}\)-ATPase of the type B intercalated cell provides the active transport step for apical Cl\(^{-}/\)HCO\(_3\)\(^{-}\) exchange (12), angiotensin II might stimulate Cl\(^{-}/\)HCO\(_3\)\(^{-}\) exchange through increased plasma membrane H\(^{+}\)-ATPase abundance, thereby increasing intracellular HCO\(_3\)\(^{-}\) concentration, which should stimulate apical Cl\(^{-}/\)HCO\(_3\)\(^{-}\) exchange (49). To explore these possibilities, CNTs were perfused in vitro in the presence or absence of angiotensin II and then fixed and labeled for pendrin and H\(^{+}\)-ATPase. Pendrin and H\(^{+}\)-ATPase subcellular distribution were quantified by immunogold cytochemistry with morphometric analysis. In response to angiotensin II application, however, we observed no increase in plasma membrane pendrin or H\(^{+}\)-ATPase abundance in type B intercalated cells (54). Therefore, this peptide hormone does not increase pendrin-dependent Cl\(^{-}\) absorption in vitro through pendrin subcellular redistribution or by increasing the driving force for this anion exchange through increased basolateral plasma membrane H\(^{+}\)-ATPase abundance. This peptide hormone may instead increase Cl\(^{-}\) absorption by acting on a Cl\(^{-}\) transporter in the CCD, other than pendrin, or may increase the driving force for apical Cl\(^{-}/\)HCO\(_3\)\(^{-}\) exchange by reducing intracellular Cl\(^{-}\) or by increasing intracellular HCO\(_3\)\(^{-}\) concentration by stimulating or inhibiting another H\(^{+}\), OH\(^{-}\), or Cl\(^{-}\) transport pathway.

While angiotensin II did not increase basolateral plasma membrane H\(^{+}\)-ATPase abundance in type B cells, it increased apical plasma membrane H\(^{+}\)-ATPase expression in type A intercalated cells nearly threefold through subcellular redistribution (54). Angiotensin II application also increased net absorption of HCO\(_3\)\(^{-}\) in the CCD, which is consistent with the observed increment in apical plasma membrane H\(^{+}\)-ATPase abundance (54). Therefore, angiotensin II applied in vitro upregulates type A intercalated cell apical plasma membrane H\(^{+}\)-ATPase expression in the CCD, similar to previous observations in the outer medullary collecting duct (54, 61).

Angiotensin II applied in vitro increases ENaC-mediated Na\(^{+}\) absorption in the CCD through a mechanism that is dose dependent and \(\text{AT}_{1a}\) receptor mediated (7, 8, 57). By stimulating ENaC-mediated Na\(^{+}\) absorption, angiotensin II should increase the lumen-negative voltage. However, our studies showed that angiotensin II increases Cl\(^{-}\) absorption without a detectable change in \(V_T\). Therefore, movement of another ion must shunt the ENaC-mediated current. Angiotensin-II-stimulated H\(^{+}\) secretion and Na\(^{+}\) absorption may shunt the voltage generated by each other, since the apical H\(^{+}\)-ATPase reduces, whereas ENaC-mediated Na\(^{+}\) absorption increases, the lumen-negative \(V_T\) (47).

In vivo angiotensin II regulates pendrin by acting on the angiotensin type 1 receptor to shift pendrin immunolabel from the subapical space to the apical plasma membrane, independently of aldosterone (83). However, angiotensin II application in vivo also activates the angiotensin type 2 receptor, which acts through nitric oxide to reduce pendrin protein abundance without changing pendrin subcellular distribution (83). Therefore, the angiotensin type 2 receptor provides a feedback loop that modulates the effect of angiotensin II-induced angiotensin type 1a receptor activation on apical plasma membrane pendrin abundance.
Regulation of Pendrin by the Mineralocorticoid Receptor Through the Interaction of Aldosterone and Angiotensin II

Aldosterone acts on the mineralocorticoid receptor (MR), which triggers a series of downstream signaling events that increase collecting duct transporter activity, such as ENaC. In the classic pathway, the MR stimulates Sgk1, which phosphorylates the ubiquitin ligase, NEDD4-2 (6). When Sgk1 phosphorylates NEDD4-2, this ubiquitin ligase cannot associate with its target transporter, which increases total and plasma membrane transporter abundance (6).

In addition to aldosterone, cortisol and corticosterone are also MR substrates. However, if a cell expresses 11β-hydroxysteroid dehydrogenase type II, cortisol and corticosterone are oxidized and thereby inactivated, making aldosterone the selective MR ligand (35). Localization studies have shown that the MR is expressed in rat and mouse type B and non-B intercalated cells (1, 70). 11β-Hydroxysteroid dehydrogenase type II is expressed in human type B and non-B, non-B intercalated cell subtypes and is also expressed within type A intercalated cells, but at lower levels (35). Therefore, within type B and non-B, non-B intercalated cells, aldosterone is most likely the preferred MR substrate. Because chemical inhibitors of the MR, such as spironolactone, reduce intercalated cell pendrin abundance (70), spironolactone may reduce pendrin abundance from a direct effect of MR inhibition or due to an indirect effect of MR blockade, such as the increase in serum potassium that follows MR inhibitor administration (48).

Shibata et al. (70) reported a novel MR phosphorylation site that is expressed exclusively within mouse intercalated cells. S843 phosphorylation prevents aldosterone binding to and thereby activating the MR. In response to dietary NaCl restriction, however, the intercalated cell MR is dephosphorylated at S843, which activates the MR, thereby increasing pendrin and H^+^-ATPase abundance within intercalated cells. Aldosterone-induced S843 MR dephosphorylation is enhanced with angiotensin II, but inhibited with hyperkalemia (70). However, more work is needed to clarify the physiological significance of this intercalated cell MR phosphorylation site. For example, whether this phosphorylation site regulates pendrin in type B and/or non-A, non-B cells remains to be determined. This could be significant, since while both angiotensin II and aldosterone upregulate pendrin, these hormones have distinct effects on pendrin abundance and subcellular distribution within these intercalated cell subtypes (82, 83, 87).

Does Pendrin Modulate Blood Pressure Solely Through Its Action in the Kidney?

Blood pressure is regulated not only by the kidney but also through the central nervous system vis-à-vis the autonomic and sympathetic nervous system, through vasoconstriction or vasodilation of vascular tissue, and through the adrenal gland by way of the production of hormones that regulate blood pressure, such as catecholamines and aldosterone (23). Further studies by our laboratory explored whether pendrin is expressed in one of these extrarenal tissues and whether it might regulate blood pressure through its action at one of these other sites. Whether pendrin protein is expressed in neural tissue is unexplored. However, pendrin is expressed in epinephrine- and norepinephrine-producing chromaffin cells within the rodent adrenal medulla, where it modulates catecholamine release (36). Although basal levels of plasma epinephrine and norepinephrine concentrations are similar in wild-type and pendrin null mice, the concentration of both of these catecholamines is higher in pendrin null than in wild-type mice after 20 min of immobilization stress.

Due to the pressor effect of catecholamines, further experiments measured blood pressure in pendrin null mice before, during, and after immobilization stress. Under basal conditions, blood pressure was lower in pendrin null than in wild-type mice, as we reported previously (50). With immobilization stress, blood pressure rose in both groups, although blood pressure remained lower in the pendrin null than in wild-type mice during the period of stress (36). However, 30 min after the relief of stress, blood pressure was similar in wild-type and in pendrin null mice (36). As such, the increase in catecholamine release observed with pendrin gene ablation may limit the fall in blood pressure observed in the absence of renal pendrin-mediated Cl^- absorption.

Since blood pressure is the product of cardiac output and systemic vascular resistance, we explored the effect of pendrin gene ablation on vascular tone (75). While we did not observe pendrin expression in vascular tissue, pendrin gene ablation increased thoracic aorta contractile force in response to either α-adrenergic agonists (phenolamine) or angiotensin II (75). This increment in contractile force is associated with increased abundance of myosin light chain kinase 20 and is eliminated with the administration of angiotensin type 1a receptor inhibitors (candesartan) in vivo (75). Since epinephrine and norepinephrine are α-adrenergic agonists, the increased vascular contractility observed in response to α-adrenergic agonists and angiotensin II may limit the hypotension observed in pendrin null mice. However, because pendrin is expressed at low levels in vascular tissue, such as the thoracic aorta, pendrin gene ablation likely enhances the force of contraction through an indirect effect, such as through changes in circulating levels or angiotensin II and/or catecholamines (75).

Because of its actions outside the kidney, pendrin may modulate blood pressure beyond its role in renal tubular transport. Since previous studies examining pendrin physiology have employed mice with global, embryonic pendrin (Slc26a4) gene ablation, these studies cannot exclude the possibility some of the phenotypic characteristics observed in these mutant mice might be from hormonal and/or neurotransmitter changes that occur during development. Examination of these potentially confounding variables will require the development of an inducible, tissue-specific pendrin null mouse.

Conclusions

ENaC and pendrin both localize in different cell types within the CNT and CCD. Angiotensin II and aldosterone increase renal NaCl absorption in part by stimulating pendrin and ENaC abundance and function in tandem. In the kidney, pendrin modulates ENaC abundance and function by increasing luminal HCO_3^- concentration and by reducing luminal ATP. However, pendrin is also expressed outside the kidney in tissues that modulate blood pressure, such as the adrenal medulla. It remains to be determined whether pendrin changes blood pressure, at least in part, through its action in these other tissues.
ROLE OF PENDRIN IN BLOOD PRESSURE REGULATION

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