Sodium and calcium transport pathways along the mammalian distal nephron: from rabbit to human

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Loffing, Johannes, and Brigitte Kaissling. Sodium and calcium transport pathways along the mammalian distal nephron: from rabbit to human. Am J Physiol Renal Physiol 284: F628–F643, 2003;10.1152/ajprenal.00217.2002.—The final adjustment of renal sodium and calcium excretion is achieved by the distal nephron, in which transepithelial ion transport is under control of various hormones, tubular fluid composition, and flow rate. Acquired or inherited diseases leading to deranged renal sodium and calcium balance have been linked to dysfunction of the distal nephron. Diuretic drugs elicit their effects on sodium balance by specifically inhibiting sodium transport proteins in the apical plasma membrane of distal nephron segments. The identification of the major apical sodium transport proteins allows study of their precise distribution pattern along the distal nephron and helps address their cellular and molecular regulation under various physiological and pathophysiological settings. This review focuses on the topological arrangement of sodium and calcium transport proteins along the cortical distal nephron and on some aspects of their functional regulation. The availability of data on the distribution of transporters in various species points to the strengths, as well as to the limitations, of animal models for the extrapolation to humans.

thiazide-sensitive sodium chloride cotransporter; amiloride-sensitive epithelial sodium channel; epithelial calcium channel; rat; mouse

In the last decade, the major salt and water transport proteins in the renal distal nephron have been identified, and their precise intrarenal localizations were explored by immunomethods or by in situ hybridization. The resulting data confirmed that the inventory of the transport proteins in the distal nephron identified so far is the same in all investigated mammalian species (rabbit, rat, mouse, and human). However, the transporter topology along the distal convolution shows subtle species differences, the unawareness of which might have been the cause for occasional discrepancies in the interpretation of experimental data. Furthermore, under altered functional conditions the abundance and extension along the distal nephron and intracellular localization of transporter proteins change. The studies also unveiled the strikingly consistent link between the given inventory of apical transport proteins along the distal nephron and the fine structure of the respective cells. This points to the potential of structural approaches in investigating functional mechanisms in distal nephron electrolyte transport in vivo.

The detailed physiology of the transporters and their involvement in human pathophysiology has been discussed in depth in excellent recent reviews (33, 44, 53, 95, 107, 110, 114, 138). Here, we will review the distributions in the distal nephron of the major sodium, calcium, and water transport proteins and regulation of their related functions, in correlation with the structural organization of the distal nephron, with the main emphasis on the distal convolution.

CORTICAL DISTAL NEPHRON

Microanatomic Organization of the Cortical Distal Nephron

The anatomic definition of the distal nephron takes into account exclusively those tubular portions that originate from the metanephric blastema: the distal straight part [thick ascending limb of Henle’s loop (TAL)], located in the medullary rays, and the convoluted portion, the “distal convolution,” located in the cortical labyrinth. The functionally defined “distal nephron” also includes, besides the TAL and the distal convolution, the cortical collecting duct (CCD), which embryologically originates from the most peripheral branchings of the ureteral bud.

Microanatomically, the distal convolution of superficial nephrons is a simple tube, opening into the most peripheral extensions of a CCD (70). In contrast, distal tubules of deeper nephron generations merge to so-called “arcades” (103). The extent of nephron fusion...
and the ratio between nephrons draining individually or through an arcade into a CCD vary among and within species (35, 103). The arcades ascend within the cortical labyrinth, proximate to the cortical radial vessels, before they open into a CCD within the medullary rays (35, 57). The number of nephrons drained by each CCD averages 11 in the human kidney (98), 6 in rabbits (58) and rats (69), and 5 in mice (71).

The present conventionally used subdivision of the distal convolution into the so-called “distal convoluted tubule” (DCT) and the “connecting tubule” (CNT; including arcades) is based on more or less quite obvious structural differences along the distal convolution. They were initially observed by Schweigger-Seidel in 1865 (118) and disclosed in microdissected preparations of kidneys from rabbits, humans, mice, sheep, cats, pigs, cattle, and dolphins in astounding detail by Peter and Inouye in 1909 (103). Some 70 years later, Peter’s light microscopic observations were confirmed by detailed electron microscopic studies in rabbits (58) and extended to rats (29).

In all species, the TAL epithelium changes at various distances downstream of the macula densa abruptly to the DCT, which is the initial segment of the distal convolution. In rabbits, the entire length of the DCT epithelium is composed by one cell type, the DCT cells. An exceedingly high density of mitochondria, encased in narrow palisade-like-arranged, interdigitated lateral cell processes, characterizes them. The DCT cells are abruptly replaced by the “CNT” cells, which by light and electron microscopy appear “lighter” and display among other irregularly arranged basolateral plasma membrane infoldings and fewer mitochondria than do DCT cells (58). Intercalated cells do not show up before the transition to the CNT and continue all along the CNT and CCD (58). In deep and intermediate nephrons, the change from the DCT to the CNT epithelium regularly occurs a few cells before fusion of two tubules; hence the arcades are entirely made up of CNT epithelium (58). The transition from the CNT to the CCD is given by the abrupt substitution of CNT by CCD cells (principal cells). Basolateral membrane infoldings in CCD cells are restricted to the most basal cell portion, and the few mitochondria are normally situated in the cytoplasm above the infoldings (58).

In rats and mice, the situation is different. On the basis of serial 1-μm sections, Crayen and Thoenes (29) reconstructed the distal convolution of a superficial rat nephron, from its beginning shortly downstream of the macula densa to the first confluence with another tubule. They distinguished by light microscopy and ultrastructure a total of four cell types; types 1–3 correspond to DCT, CNT, and CCD (principal) cells and type 4 to intercalated cells. Type 1 (DCT) cells exclusively comprised the first part of the tubular portion. In the direction of the flow, these cells became intermingled with intercalated cells. Then, type 2 cells progressively replaced type 1 cells, and type 3 cells progressively replaced type 2 cells. Furthermore, cell height, basolateral cell membranes, and mitochondrial density of cell types 1–3 gradually decreased in flow direction. Because of the gradual structural changes, Crayen and Thoenes concluded that in rats segmentation of the distal convolution can be made only arbitrarily. A decade later, the lack of sharp segment borders in the rat distal convolution was confirmed by Dorup (35), and excellent ultrastructural descriptions of each of the four cell types were given by Madsen and Tisher (83). Dorup (35) also reported, in addition to the gradual segment transitions, ultrastructural distinctions between the DCT cells in the early and late part of the DCT. These were corroborated by the observation of the abundant presence of caveolin in late DCT cells (17), but not in early DCT cells, consistent with the structural abundance of caveolae on the basolateral plasma membranes of cells in this tubular region (57).

In mice (Fig. 1), the distal convolution seems to be similarly organized as it is in rats; however, systematic ultrastructural studies are not available. Ultrastructural descriptions of the transitional regions in the human distal convolution are lacking as well.

Distribution of Transport Proteins Along the Distal Nephron

The following apical transport systems have been shown by immunomethods and/or in situ hybridization to be confined in the kidney exclusively to the distal nephron: the bumetanide-sensitive sodium–2 chloride potassium cotransporter (NKCC2) (62, 82, 97), the thiazide-sensitive sodium–chloride cotransporter (NCC) (3, 96, 105), the amiloride-sensitive epithelial sodium channel (ENaC) (37), the recently discovered epithelial calcium channel (ECaC1, CaT2; TRPV5) (54, 101), and the vasopressin-sensitive water channel [aquaporin-2 (AQP2)] (66, 94). The basolateral sodium/calcium exchanger (NCX) (96, 108) and the plasma membrane Ca-ATPase (PMCA) (13, 14), as well as the cytoplasmic calcium-binding protein calbindin D28k (10, 13, 112) are, in contrast to other nephron portions, particularly abundant in some parts of the distal nephron.

Mapping the apical transport systems along the distal nephron of rabbits (77), rats (26, 55, 116), mice (19, 65, 78), and humans (11) revealed their serial arrangement. NKCC2 is confined to the TAL, including the macula densa (62, 82, 97), and distinguishes this segment from all others and in all species. Salt subtraction from the tubular fluid via NKCC2 in the (water impermeable) TAL is the precondition for urinary concentration. Solute reabsorption by the subsequent distal convolution is the premise for fine-tuning of renal electrolyte excretion. It proceeds by the concerted action of the apical transporters NCC, ENaC, ECaC1, and AQP2, as well as others not mentioned in this context. Of these transporters, NCC is without exception the most upstream transporter in the distal convolution and replaces the NKCC2 exactly at the structural transition from the TAL to the DCT (3, 96, 97, 105) (Fig. 1, a–c).

What differs markedly among the species is the site of start-off along the distal convolution of ENaC, ECaC1, and AQP2 expression. In the rabbit kidney
Fig. 1. Mouse cortical distal nephron portions. a, d, and g: 1-μm Epon sections. b, c, e, f, h, and i: Cryostat sections. a–c: Arrows, transition from the thick ascending limb (T) to the early distal convoluted tubule (D1); P, proximal tubule. b and c: Double immunofluorescence for bumetanide-sensitive sodium-2 chloride potassium cotransporter (NKCC2) and thiazide-sensitive sodium-chloride cotransporter (NCC). d–f: Approximate transition from the late distal convoluted tubule (DCT; D2) to the connecting tubule (CN). e and f: Double labeling for H^+ATPase, prominent in intercalated cells, and aquaporin-2 (AQP2), confined to connecting tubule cells. g–i: Transition from CN to the cortical collecting duct (CD) in the medullary rays. h and i: Consecutive cryostat sections stained for epithelial sodium channel (ENaC) and AQP2. Bars = ∼50 μm.
(Fig. 2, top; Fig. 4, a and b), immunostaining for ENaC starts where in situ hybridization for NCC mRNA stops (77). NCC immunostaining, made with antibodies directed against a synthetic peptide, is replaced abruptly by ECaC1 immunostaining (Fig. 4, a and b; Lofling J and Lofling D, unpublished observations). Immunostaining with a monoclonal antibody against a metolazone-binding protein continues beyond the stop of the in situ signal for some distance into the CNT (3), where it colocalizes with ECaC1 (52). The abrupt start of coexpression with ENaC of the vasopressin-sensitive water channel AQP2 (Fig. 3, a and b) sharply marks the beginning of the CCD (77), which structurally is discernible by abrupt replacement of CNT cells by CCD cells (principal cells). The basolateral NCX is immunohistochemically detectable exclusively in the CNT (3, 108), and calbindin D28K is intermediate in the DCT, rises sharply with the beginning of the CNT, and continues somewhat more weakly along the CCD (77). The congruency of structural segmentation and distribution pattern of apical transporters along the rabbit distal nephron are explicit. The distribution pattern of apical transporters along the rat distal convolution mirrors the above-described lack of sharp structural transitions in this species. DCT cells in this late portion differ in some structural aspects from those in the early portion (see above) and coexpress, in addition to NCC, the apical channels ENaC (116) and ECaC1 (55). Furthermore, they display a very high abundance of cytoplasmic calbindin D28K (76, 105) and basolateral NCX (96). In male Sprague-Dawley rats, the fractional length of the late NCC-displaying tubular portion, defined by its high abundance of basolateral NCX, has been estimated to amount to ~20% of the total NCC-positive portion (26).

A further significant difference between rats and rabbits concerns the distribution of AQP2 in the distal nephron. In rats, immunostaining for AQP2 has been detected to start with the break-off of NCC-staining (77). Occasionally, AQP2-positive cells may be intermingled even with the last few NCC-positive cells. This observation agrees with the former structural data on intermingling of cell types along the distal convolution (29, 35). Quantitative estimation by Western blot analysis of AQP2 in preparations of isolated rat CNT and CCD revealed ~60% AQP2 abundance in the CNT from that in the CCD (66), consistent with weaker AQP2 immunostaining along the CNT than in the CCD of rats (26, 66, 77). The CCD cells in the successive segment coexpress ENaC and AQP2, but not ECaC1. In the cortex of male Sprague-Dawley rats, the fractional volumes of DCT, CNT, and CCD correspond to ~1:1:1 (26).
In mice, the distribution of NCC, ENaC, ECaC1, and AQP2 (Fig. 1, c and f; Fig 2, middle; Fig 3, c and d; Fig. 4, c and d) resembles that in rats. In the late part of the DCT, the apical transporters NCC, ENaC, and ECaC1 are coexpressed (78) and, additionally, the most downstream NCC-positive cells might even coexpress AQP2 (Loffing J, unpublished observations). A significant abundance of cytoplasmic calbindin D28k, basolateral NCX (19, 78), and PMCA (78) has also been demonstrated by immunomethods in the early portion of the DCT. Nevertheless, a marked jump in abundance of these latter proteins was consistently observed at the sites where, in addition to NCC, coexpression of ENaC and ECaC1 began (Fig. 5) (78). The functional link between apical ECaC1 and NCX and PMCA is further evidenced by the parallel decreases in apical ECaC1 and basolateral NCX and PMCA immunostaining along the CNT and their simultaneous cessation at the histotopographically recognized CCD (78). Single cells, coexpressing ECaC1, NCX, PMCA, and calbindin D28k, are occasionally interspersed in the CCD epithelium (78). In female C57/BL6 mice, the fractional volumes of early DCT, late DCT, and CNT correspond to 1:110:112:1 (75).

In humans, the tubular portion with overlap of NCC and ENaC is rather short. NCC characterizes ~30–35% of profiles of the distal convolution and ENaC ~70–75% (11). AQP2 does not directly supersede the NCC, but the initial ~15% of the CNT [at least the portions before the fusion with arcades display ENaC alone (Fig. 3, e and f)] (11). The distribution of ECaC1 along the human nephron has not been assessed by immunostaining so far. Similarly to mice, NCX, PMCA, and calbindin D28k are traceable to varying extents even in the initial portion of the DCT, and, in pronounced difference from all other species, continue in significant abundance along the cortical collecting duct (11).

**Definition of the Distal Nephron Based on Distribution of Transport Proteins**

The clear-cut structural and functional organization of the rabbit distal nephron constitutes a simple model, which aids in an understanding of the more complex distal nephron organization in rats, mice, and humans (Fig. 6). In all four mammalian species analyzed so far, of the proteins discussed in this article, NKCC2 is the most upstream apical salt transporter and unequivocally defines the TAL. The succeeding salt transporter is the NCC, the onset and end of which in the distal

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**Fig. 3.** Farthest upstream sites of AQP2 in the distal nephron of a rabbit, mouse, and human. All sections are cryostat sections showing immunofluorescence. a and b: Rabbit sections (modified from Ref. 77). Shown are double labeling for calbindin D28k (CB) and AQP2, respectively, and abrupt start of AQP2 immunostaining, coincident with sharp decrease of CB immunostaining at the sharp transition (arrowheads) from CN to CD. D, DCT. c and d: Mouse (unpublished observations). Shown are double labeling for NCC and AQP2, respectively, intermingling of NCC- and AQP2-positive cells (arrowheads) at the end of DCT (D2), and beginning of the CN, where NCC disappears. e and f: Human consecutive cryostat sections (modified from Ref. 11) labeled for ENaC and AQP2, respectively. Shown is the lack of AQP2 immunostaining in most upstream ENaC-labeled CN profiles (CN*). Bars = ~ 50 μm.
convolution of all species are clearly discernible, and which defines the DCT. The next apical ion transporters in the series are ENaC and ECaC1. In rabbits, they abruptly replace NCC in the apical membrane. In rats, mice, and humans, ENaC and ECaC1 seem to be “pushed” more or less upstream along the distal convolution into the NCC-displaying late portion of the DCT, giving rise to a portion with apical coexpression of NCC, ENaC, and ECaC1. Accordingly, in these latter species the DCT can be further subdivided into an early and a late portion. Besides the coexpression with NCC of the apical transporters ENaC (11, 79, 116) and ECaC1 (55, 78), the second portion differs from the first, e.g., by the presence of intercalated cells (35, 83), by very prominent cytoplasmic calbindin D28k (76, 105), by basolateral NCX (96) and PMCA (78), as well as by discrete structural differences (35). The conspicuous onset of basolateral NCX immunostaining in the NCC-positive segment of the rat (96) prompted the groups of Bachmann (3) and Ellison (40) to propose the subdivision of the DCT into DCT1 (NCX negative) and DCT2 (NCX positive). According to this criterion, neither in mice (19) nor in humans could such subdivision of the DCT be made. The onset of traceability of ENaC and ECaC1, as far as has been investigated to date, coincides approximately with the marked start (rat) (96) or rise (mouse) (78) in NCX, PMCA, and calbindin D28k immunostaining. The coexpression of NCC and ENaC has been used as well to distinguish the late (~DCT2) from the early (only NCC expressing) DCT (~DCT1) (78). That the late part of the DCT varies in length among species, probably also among species strains, possibly along with age, sex, and other factors, has been emphasized in an editorial comment by Wade (134).

The end of NCC immunostaining can be used in all species to mark the beginning of the CNT. In rabbits, it comes along with the onset of ENaC and ECaC1 and basolateral NCX immunostaining and in rats and mice with onset of additional AQP2 coexpression (see Fig. 6). In all species, the presence of AQP2 and the histotopographical location of the tubule in the medullary ray (or in superficial nephrons the proximity to the renal capsule) defines a CCD. The overall length of the distal convolution (DCT and CNT) can be conveniently estimated by double immunostaining for NCC and NCX (26, 96) or NCC and calbindin D28k (11).
Relevance of Specific Transporter Topology

The specific sequence of electrolyte transporters along the distal nephron probably guarantees sodium recovery under a large range of physiopathological situations. It seems to imply the possibility of partially compensating for inadequate salt reabsorption by adapting salt reabsorption via other and differentially regulated salt transport systems in downstream tubular portions. For instance, impairment of NaCl reabsorption in the TAL, by whatever means, drastically increases the NaCl load in the downstream segments of the TAL, which respond with higher salt reabsorption rates, and over longer periods, with increased transport capacity and associated epithelial hypertrophy (70). Such compensatory mechanisms along the nephron are thought to occur also under impaired sodium uptake due to mutations of genes for some transport proteins (e.g., NKCC2 in Barter syndrome, NCC in Gitelman syndrome) (49, 107).

Although at first glance the species differences in the distribution pattern of transporters appear to be rather trivial, their functional relevance might be substantial. The definite (rabbit) or gradual (rat, mouse, human) changes in the distribution of specific transport pathways along the distal nephron are also reflected in respective distribution patterns of sensitivities to various peptide hormones (87). For instance, the sites of vasopressin sensitivity along the nephron are thought to occur also under impaired sodium uptake due to mutations of genes for some transport proteins (e.g., NKCC2 in Barter syndrome, NCC in Gitelman syndrome) (49, 107).

Thus the final result of solute excretion depends not only on the presence and abundance of given transport proteins in the kidney but also on their species-specific topological arrangement.

CONTROL OF TRANSPORT ACROSS APICAL PROTEINS

The differences in the specific distribution patterns of transport proteins are probably evolutionary responses to species-specific living conditions. Evidently, within this given frame there is room for individual adaptation to the actual needs. Immunomethods and/or morphology can disclose the nephron sites in which, under specific functional conditions, in vivo adaptive changes took place.

Regulation of NCC-Mediated Salt Reabsorption

NCC mediates electroneutral NaCl uptake into the DCT cells. Because renal NCC is confined to the DCT, data on NCC derived from whole-organ homogenates truly reflect corresponding changes in NCC abundance in this segment. As long as specific anti-NCC antibodies were not available, abundance of NCC was often assessed by its specific binding to metolazone, a thiazide-like diuretic (5). Changes in metolazone binding protein, NCC abundance, or NCC mRNA have been observed under many different pathophysiological or clinical situations (for a review, see Refs. 47, 67, and 107). What might be the common denominator under the differential conditions, controlling specifically apical NCC abundance and NCC-mediated cellular salt uptake?
Tubular sodium load and flow rates. Some data from the time when neither [3H]metolazone-binding studies nor NCC antibodies were available suggested that tubular salt load and/or flow rate in the DCT directly controls sodium reabsorption in the DCT. Rabbits under chronic high-sodium, reduced-potassium diets (with respective chronic very low endogenous plasma levels of aldosterone) revealed marked hypertrophy of the DCT epithelium, indicating chronically upregulated transport capacity in the DCT epithelium (59). Micropuncture studies in rats under furosemide-induced impairment of NaCl reabsorption (with simultaneous replacement of salt and fluid loss) confirmed that the marked increases in surface basolateral plasma membrane and in mitochondrial volume in the distal convolution cells mirror correspondingly increased sodium reabsorption rates (40, 60, 122). In situ hybridization (96) as well as RNAse protection assays (140) disclosed upregulation of mRNA for NCC under the given conditions. Others were unable to detect any increase for NCC mRNA (1, 88). Indications for NCC mRNA-independent regulation of NCC protein abundance, e.g., by mechanisms that control protein translation and/or stability, have been obtained in some studies (1, 137), but so far intracellular processing and trafficking of NCC in vivo have not been addressed. Studies in rats under the above experimental conditions demonstrated the involvement of IGF-1 and IGF binding protein 3 in the hypertrophy of the DCT epithelium (68).

Steroid hormones. MINERALOCORTICOIDS AND GLUCOCORTICOIDS. In rabbits, drastically increased endogenous aldosterone levels associated with chronic low-sodium, high-potassium intake did not increase the transport machinery in the DCT but did markedly in the CNT (59). Adaptive structural and functional hypertrophy in the DCT epithelium after furosemide-induced inhibition of salt reabsorption in the TAL occurred in adrenalectomized rats with clamped plasma levels of mineralocorticoid and glucocorticoid hormones to similar extents as in intact rats (60, 122). These collected observations suggested that changes in salt delivery and flow rate by themselves might be sufficient to initiate modulation of salt transport rates in the DCT. Nevertheless, several studies advocated the implication of aldosterone in the control of apical NCC abundance and/or NCC-mediated salt reabsorption. In rat kidneys, low dietary sodium intake, which disclosed upregulation of mRNA for NCC under the given conditions. Others were unable to detect any increase for NCC mRNA (1, 88). Indications for NCC mRNA-independent regulation of NCC protein abundance, e.g., by mechanisms that control protein translation and/or stability, have been obtained in some studies (1, 137), but so far intracellular processing and trafficking of NCC in vivo have not been addressed. Studies in rats under the above experimental conditions demonstrated the involvement of IGF-1 and IGF binding protein 3 in the hypertrophy of the DCT epithelium (68).

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MINERALOCORTICOIDS RECEPTORS AND 11β-HYDROXYSTEROID DEHYDROGENASE TYPE 2. Canonically, a direct role of mineralocorticoid hormones in the control of apical NCC abundance and of NCC-mediated NaCl transport requires the presence in the DCT of mineralocorticoid receptors (MR) and of the enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), which confers mineralocorticoid specificity to the MR by the rapid metabolism of glucocorticoids. MR and 11β-HSD2 have been revealed by various methods in the distal nephron of rats, rabbits, and humans (for a review, see Refs. 42 and 107) and 11β-HSD2 also in mice (19). Whereas MR seem to be present all along the rat DCT and CNT (15), 11β-HSD2 is not detectable in the early (ENaC-lacking) portion (DCT1) but is well detectable in the second DCT portion (DCT2), in the CNT, and CD (15, 116). These observations indicate that the effects of aldosterone on NCC-mediated sodium transport might differ along the axis of the DCT, and they corroborate the suggestion that the DCT may exhibit some promiscuity with respect to the selectivity for mineralocorticoids and glucocorticoids (107). Reilly and Ellison (107) proposed that the latter may stimulate NCC-mediated sodium transport primarily in the early DCT (DCT1), whereas aldosterone may stimulate sodium transport predominantly in the late DCT (displaying NCC and ENaC), where MR and 11β-HSD2 are both highly expressed. Putatively, control of NCC abundance by aldosterone via MR-independent noncanonical pathways is conceivable.

SEX HORMONES. Sex hormones seem to affect directly or indirectly NCC-mediated salt transport. RT-PCR (74) and autoradiographic (30, 124) experiments pointed to the expression of estrogen receptors in rat kidneys. Chen et al. (23) and Verlander et al. (130) demonstrated that estrogens given to ovariectomized rats increase [3H]metolazone binding sites and NCC abundance, respectively, in the renal cortex. These observations were used to explain why female rats respond with a more pronounced diuresis to thiazides than do male rats (23). Whether increased NCC-mediated sodium transport contributes to the sodium retention seen under estrogen therapy (e.g., the contraceptive pill) is not known.

Peptide hormones. The presence of receptors for peptide hormones such as calcitonin, PTH, and isoproterenol had been shown indirectly by the increases in cAMP activity after application of these hormones to morphologically defined, microdissected distal tubule segments (87). Blakely et al. (12) reported increased [3H]metolazone binding in kidney homogenates of calcitonin-treated rats.
Angiotensin II is involved in the control of arterial blood pressure and whole body sodium homeostasis. Independently from its effect on adrenal aldosterone secretion, angiotensin II stimulates sodium reabsorption in the distal tubule (135). It has been discussed that the hypertrophy in the DCT under increased salt load of the DCT (after furosemide treatment, combined with high salt and water load) might be ascribed, in part, to an effect of angiotensin II (6). The role of angiotensin II in NCC regulation can be deduced from recent experiments in mice with targeted disruption of the angiotensin II type 1 (AT1) receptor (18). Unlike wild-type mice, these AT1 knockout mice lack increases in NCC protein abundance in response to a low dietary sodium intake. Thus in the rodent DCT part of the adaptation to low dietary sodium intake is possibly mediated by increased angiotensin II levels.

Inhibition of NCC-mediated transport. Thiazide diuretics are frequently used in the treatment of hypertension. Thiazides inhibit the uptake of NaCl into the DCT cell by binding to NCC. Prolonged reduction of sodium entry into the cells results in chronically lower transport rates, structurally reflected by lowering of the epithelium with a reduction of the active-salt transporting machinery (70). In the DCT of mice, treatment with thiazides is indeed associated with lowering of DCT epithelium (Valderrabano V and Loffing J, unpublished observations), and NCC knockout mice have a marked hypotrophy of the DCT epithelium (117). However, treatment of rats with metolazone for 3 days or with hydrochlorothiazide induced massive apoptosis exclusively in the early DCT (76). In rats treated continuously for 2 and 4 wk with metolazone, the epithelium of the early DCT was markedly simplified, displaying fewer basolateral membranes and mitochondria, and de- and regenerating cells. These observations suggest chronically lower transport rates and corroborate former data by Morsing et al. (89), who measured that post-chronic in vivo blockade of NaCl transport by thiazides reduced transport capacity of rat distal tubules, despite a significantly increased number of thiazide binding sites. The authors pointed out that increases in the number of thiazide receptors are not necessarily synonymous with increases in transport activity. The different effects of thiazides on the DCT in rats (apoptosis) and mice (hypotrophy) once again emphasize species differences, even between closely related species.

Regulation of ENaC-Mediated Sodium Transport

The second major sodium transporter in the distal convolution is ENaC. ENaC is the major player in aldosterone-regulated sodium reabsorption, not only in the kidneys but also in other organs (e.g., distal colon, salivary glands) (131). In the kidney, all ENaC-positive tubule portions have been subsumed under the term “aldosterone-sensitive distal nephron” (ASDN) (81), which comprises the CNT and CD in all species and includes, in some species at least, the late part of the DCT (see above). The essential role of ENaC in control of salt and volume homeostasis is highlighted by the fact that some hereditary forms of severe salt-sensitive arterial hypertension (Liddle’s syndrome) and severe renal salt wasting (pseudohypoaldosteronism type I) are related to gain- and loss-of-function mutations, respectively, of ENaC genes (for a review, see Ref. 110).

Sodium uptake into the cell via ENaC is electrogenic and can be measured by corresponding sodium currents that are inhibited by amiloride. ENaC-related sodium transport favors potassium secretion, which proceeds most probably via the apical renal outer medulla potassium channel ROMK, coexisting with ENaC in the same cells (136). Putative potassium reabsorption by intercalated cells, which are regularly interspersed among the ENaC-expressing cells, might modify the rigid link between sodium reabsorption via ENaC and K secretion via ROMK.

ENaC is composed of three subunits (α, β, γ) (110). All three subunits have been located along the ASDN (37), although in differential immunohistochemical abundance and intracellular sites (50). Interestingly, α-ENaC, but not β- and γ-ENaC, has been evidenced by RT-PCR in microdissected rabbit DCTs; however, amiloride-sensitive sodium currents could not be detected in this segment (129). Expression of γ-ENaC mRNA has been also revealed by in situ RT-PCR in the mouse TAL and DCT (25).

Regulation of renal sodium transport via ENaC could intervene 1) directly at the level of single channels in the membrane, by changing the channel gating kinetics; in vitro studies in amphibian cells and heterologous expression systems suggested this possibility (for a review, see Refs. 38 and 48), but to our knowledge, such effects have not been described so far for the ASDN in vivo; or 2) at the level of density of functional apical channels. This can result either from altered synthesis rates of ENaC subunits and/or from altered exo- and endocytosis rates of already present ENaC subunits.

Role of synthesis of ENaC subunits. Although the exact stoichiometry of the subunits in the channel is still debated, there seems to be consensus that the α-subunit plays a pivotal role in the assembling of functionally active channels (120). In the kidney, primarily the abundance of α-ENaC appears to be regulated by aldosterone. Exogenous aldosterone application increases the abundance of α-ENaC at the mRNA and protein level (84, 123). Dietary sodium restriction, which increases endogenous aldosterone production, has been shown to induce α-ENaC in some (84, 85, 140) but not all studies (109, 123). Based on previous studies in Xenopus laevis A6 cells, it has been proposed that the induction of α-ENaC might be a prerequisite for the apical translocation of ENaC (86). Sufficient availability of α-ENaC may allow full assembly of ENaC channels and their subsequent release from the endoplasmic reticulum and delivery to the cell surface. The induction of α-ENaC (at least in nonadrenalectomized rats) in response to aldosterone, however, is rather small, and it is conceivable that in vivo the induction of α-ENaC alone does not account for the apical targeting
of all three ENaC subunits. Interestingly, Nielsen and co-workers (93) recently reported that MR inhibition by spironolactone blunts the induction of α-ENaC but does not prevent the apical redistribution of all three ENaC subunits in response to a low-sodium diet. Short-term adaptation of the kidneys to 24 h of dietary sodium restriction apparently does not involve significant upregulation of α-ENaC (85). However, amiloride-sensitive renal sodium reabsorption was shown to be significantly increased after 4 h of sodium restriction (46).

Other hormones besides aldosterone affect the synthesis rate of ENaC subunits as well, and thereby they might also intervene in the control by aldosterone of ENaC-mediated sodium reabsorption. For example, in the rat kidney in vivo chronic increases in vasopressin levels induce β- and γ-ENaC at the mRNA level (92), in agreement with studies in rat CCD cells in vitro (34). Similarly, water restriction in normal rats and chronic infusion of dDAVP (a stable analog of vasopressin) into Brattleboro rats (a rat strain that lacks endogenous vasopressin) increase β- and γ-ENaC protein abundance (39).

Angiotensin II rapidly stimulates ENaC activity in isolated distal nephron segments (135), an effect most likely mediated by AT1 receptors (104). Such acute effects of angiotensin II are unlikely to be mediated by an increased ENaC synthesis rate, but recent experiments in AT1 receptor knockout mice suggest that angiotensin II might have a direct impact on ENaC abundance as well. Despite elevated plasma aldosterone levels, the knockout mice express less α-ENaC than wild-type mice (18).

Regulation of ENaC activity by redistribution of ENaC subunits. In rodent kidneys, dietary sodium restriction causes an immunohistochemically traceable redistribution of ENaC subunits from intracellular compartments toward the apical cell surface (79, 84). These observations correlate well with previous patch-clamp studies in isolated rat collecting ducts by Pacha and co-workers (99), who recorded increases in the number of open sodium channels in the apical plasma membrane after 1 wk of dietary sodium restriction (99). The increases in active channels were in parallel to increases in endogenous plasma aldosterone levels (99) and were observable even within 15 h after sodium restriction (45). Immunohistochemistry revealed that in adrenalectomized rats within less than 4 h after one single aldosterone injection, the apical ENaC density increased (81). The rapid upregulation of apical ENaC activity and abundance indicates that such changes might be relevant not only for the long-term adaptation of renal sodium excretion but also in the adaptation to circadian variations of dietary sodium intake (45).

The molecular mechanisms underlying the rapid accumulation of ENaC in the apical cell surface are not well understood. Data suggest involvement of the serum and glucocorticoid-regulated kinase SGK1, which is an aldosterone-induced protein (20, 90). SGK1 induction by aldosterone is clearly dose dependent (119) and occurs even under small variations in endogenous plasma aldosterone levels provoked by alterations in dietary sodium intake (56). Coexpression of ENaC with SGK1 in X. laevis oocytes manifestly stimulates ENaC activity (20, 90) and cell surface abundance (32). The latter is thought to be related to SGK1-dependent phosphorylation and inactivation of the ubiquitin ligase Nedd4–2 that downregulates ENaC (31, 121). The importance and regulation of SGK1 and Nedd4–2 under various experimental conditions are summarized in several recent reviews (61, 73, 80, 100, 120).

The induction of SGK1 by aldosterone, detectable by immunomethods exclusively in the ENaC-positive DCT2, CNT, and CD cells (81), precedes the apical targeting (81) and functional activation of ENaC (9). An in vivo role of SGK1 in ENaC regulation can be deduced from recent findings made in SGK1 knockout mice (141). However, the salt-losing phenotype of the SGK1 knockout mice is mild compared with that of MR knockout mice (7) or that of the α-, β-, or γ-ENaC knockout mice (110). These observations in SGK1 knockout mice suggest that SGK1 might be an important, but not the sole, player necessary for apical ENaC activity.

Axial differences in ENaC density. Under standard conditions of sodium intake, all three subunits are traceable in the apical membranes only in the initial parts of the ASDN (77, 79). Farther downstream in particular, β- and γ-subunits seem to vanish from the apical membrane and become increasingly prominent in intracellular compartments (50, 77, 79, 84). Former studies on net sodium reabsorption in micropерfused rat tubules (27, 106, 125) and in isolated rabbit tubules (2) demonstrated that in upstream portions of the ASDN (late DCT and CNT; formerly designated together as “late distal tubule”), sodium transport was consistently present and several times higher than in CCDs located downstream. The axial decrease in the basolateral Na-K-ATPase (63) and the morphological correlate of the sodium extrusion apparatus (59), i.e., the basolateral membrane infoldings and the mitochondrial volumes, also mirrors progressive decreases in the sodium transport rates along the ASDN. The functional and morphological data match the progressive immunohistochemical decrease along the ASDN of the apical expression of ENaC. Under chronic high-salt intake, associated with low aldosterone levels, apical ENaC is barely detectable, even at the very beginning of the ASDN (79). Under moderate standard salt intake by animals in European laboratories, apical α-, β-, and γ-ENaC are found to extend at best along the DCT2 and the early CNT (77, 79). Under salt restriction, inducing rises in endogenous aldosterone plasma levels, apical ENaC is detectable also in the CNT and outer medullary CD (79).

What causes the axial differences in apical ENaC and, consequently, in sodium transport along the ASDN? Theoretically, an axially decreasing gradient of apical ENaC localization might be connected with correspondingly distributed MR density along the ASDN. However, former data by Farman et al. (43) as well as Doucet and Katz (36) showed similar densities or even
rather increasing MR densities along the ASDN in rats. Equal sensitivity for aldosterone along the collecting system is also suggested by the aldosterone-dependent induction of SGK1 and α-ENaC, which occurred uniformly all along the collecting system (81). This implies that other factors superimpose the systemic changes in aldosterone levels and contribute to the differential regulation of apical ENaC abundance along the distal nephron. Whether differential sensitivities along the ASDN for other hormones, known to affect ENaC function (e.g., insulin, vasopressin, angiotensin II), exist and whether these hormones influence ENaC surface expression are not known. Extracellular proteases [e.g., kallikrein, channel-activating protease-1 (CAP1)] (24, 126, 133) secreted into the tubular fluid might contribute to axially differential regulation of ENaC-mediated salt transport. Kallikrein is synthesized in the most upstream portions of the ASDN (132). Prostasin, the human homologue of *X. laevis* CAP1, has been recently shown to be increased in urine in humans under conditions of increased plasma aldosterone levels (91). However, the extracellular proteases seem to affect channel activity (24, 126, 133) rather than ENaC cell-surface expression.

What necessarily changes in tubular flow direction is the tubular fluid composition. Osmolarity changes and intra- and extracellular ion concentrations (e.g., for sodium, calcium) have been discussed to play a role in ENaC regulation (48). Also, effects of tubular flow rate on ENaC-mediated sodium transport in isolated rabbit collecting tubules have been reported (113).

Taken together, the marked gradient in apical ENaC density, assessed by in vivo experiments, agrees with functional data on sodium transport in different ASDN portions. The causes for this gradient along the ASDN are still unclear. The findings suggest that local factors along the tubule, presumably via the tubular fluid composition, importantly modify the effects of systemic factors on ENaC-mediated transport.

**Sites of Calcium Transport in the Distal Nephron**

Former micropuncture experiments and studies in isolated tubules of rabbits attributed active, transcellular movement of calcium to the distal segments downstream of the macula densa (reviewed in Refs. 44, 53, and 107). The observation that, in humans, the tubular portion with a high abundance of the calcium-extruding proteins PMCA and NCX, and calbindinD28k, is much longer than in rabbits, rats, and mice (see Fig. 6) suggests that, in humans, relevant transcellular calcium transport may occur over a much longer tubular portion than in laboratory animals, i.e., all along the distal convolution and also in the cortical collecting duct.

The specific apical calcium channels in the distal tubules remained elusive for a long time. Verapamil (dihydropyridine)-sensitive (cardiac L-type) calcium channels have been implicated in transcellular calcium movements (4, 72). In an immortalized mouse DCT cell line, antisense oligonucleotides directed against the α1c- or the β3-subunits of verapamil-sensitive calcium channels inhibited the rise in intracellular calcium concentrations in response to PTH or chlorothiazide (4). Targeted disruption of the β3-subunit in gene-modified mice blunted (in vivo) the hypocalciuric action of thiazides (8). These data could support the hypothesis that verapamil-sensitive calcium channels indeed may be implicated in transcellular calcium transport in the distal nephron. However, the predominantly cytoplasmic and basolateral distribution pattern of α1c-subunits of the cardiac L-type calcium channel is more compatible with a role of these channels in intracellular and membrane signaling processes rather than in transcellular calcium movement (142). The epithelial calcium channel ECaC1 exhibits highly selective calcium permeability, is activated by hyperpolarization, and yet is insensitive to verapamil (53). A homologous channel was identified from rat kidney by Peng et al. (101) and called “calcium transporter 2” (CaT2) (101), to distinguish it from the apical calcium channel CaT1 in intestine described earlier (102).

Interestingly, immunohistochemical ECaC1 traceability in the kidney of rabbits (52), rats (55), and mice (78) goes precisely in parallel with immunohistochemical abundance of PMCA and NCX in distal nephron portions. Particularly well evident is the axial decrease in ECaC1 localization in the apical membrane from the most upstream ECaC1-positive portions toward cytoplasmic domains in portions further downstream in mice (78) and the parallel decreases in basolateral NCX and PMCA (Fig. 5). These observations suggest a parallel reduction of calcium transport rates. Whether the intracellularly localized ECaC1 molecules are recruited to the apical plasma membrane, and in response to which stimulus, is unknown as yet. Like NCX (see above), ECaC1 also appears to be upregulated by estrogens (127). Consistent with a role of ECaC1 in regulated transcellular calcium transport is the response in vivo at the mRNA and protein level to vitamin D3 (55) and the reduced ECaC1 expression in kidneys of 25-hydroxyvitamin D3-1α-hydroxylase knockout mice (51). These observations, taken together, speak in favor of ECaC1 as the key apical calcium channel in renal transcellular calcium transport in vivo (53). However, the notion that NCX and other proteins involved in transcellular calcium movement are detectable, although in comparably lower abundance, in distal sites lacking ECaC1 suggests that these tubular portions and other apical transporters may contribute to some calcium homeostasis as well.

**Interaction of sodium and calcium transport along the distal convolution.** Costanzo and Windhager (27, 28) demonstrated in a series of microperfusion experiments that the acute application of thiazides or amiloride has a direct stimulatory effect on transcellular calcium reabsorption in the renal distal convolution. The obvious inverse relationship of sodium and calcium transport along the distal convolution has been explained by two rationales. 1) Ion transport inhibition by thiazide and amiloride hyperpolarizes the cells, and thereby activates calcium channels within the apical...
plasma membrane (44). The hyperpolarization of the cells is thought to be either indirectly related to the lowered chloride entry via NCC, causing an enhanced chloride influx across chloride channels, or is directly related to impaired sodium entry via ENaC, respectively. 2) The reduced apical sodium entry lowers intracellular sodium concentration and thus may increase the driving force for basolateral Na/Ca exchange (44). These mechanisms could explain the hypocalciuric effect of thiazide diuretics and amiloride, as well as the reduced urinary calcium excretion found in patients with loss-of-function mutations for NCC (Gitelman syndrome) (44, 115). However, alternative explanations for the hypocalciuria seen under these conditions are conceivable. For example, reduced urinary calcium excretion may be secondary to extracellular volume contraction, contributing to enhanced sodium and calcium reabsorption in the proximal tubules (16, 139).

CONCLUDING REMARKS

The cortical distal nephron is the site of fine regulation of renal electrolyte excretion. Along this nephron portion, three different salt transporters are arranged in series: the two cotransporters NKCC2 and NCC, and ENaC. In addition, within the distal convolution ENaC is consistently coexpressed (as far as is known) with ECaC1, and in some species also with the vasopressin-sensitive water channel AQP2. Because the transport activity of each of these transporters is differentially controlled, the serial arrangement might guarantee, teleologically speaking, optimal electrolyte recovery under a vast range of environmental conditions.

Although the inventory and the basic sequence of transporters along the distal cortical nephron are the same in kidneys of the investigated mammalian species, there exist, however, subtle differences that might be functionally relevant. The distinctions pertain to the extent of coexpression of NCC with ENaC and ECaC1, ranging from not existent in the rabbit to considerable in the mouse, and to the lengths of overlap of ENaC and AQP2. The species-specific distributions of transport proteins along the distal nephron coincide with the respective structural organization, either with sharp (rabbit) or with more or less progressive (mouse, rat human) structural segment transitions in the distal convolution.

These species differences present some problems. One is a semantic one regarding criteria for subdivision of the distal convolution. The segments DCT, CNT, and CCD are clearly demarcated by structure and congruent distribution of transport proteins only in rabbits. The more or less gradual structural changes and the partial coexpressions of transporters in the other species make segmentation a matter of definition. Thus interpretation of functional data from the distal nephron needs precise criteria defining the investigated nephron portion.

A presumably more challenging problem is implicated in the functional consequences of the different species-specific topological arrangements of the transporters. Any maneuver affecting a given transporter activity in the distal nephron will result in slightly different secondary changes along the nephron and in the final urinary electrolyte excretion pattern in each species. These differences might be amplified by the exceedingly functional plasticity of the distal convolution, in particular of the DCT, triggered by the manifold factors mentioned in this article and probably by many others not discussed here.

Thus despite the large congruence among the species with respect to the occurrence of transport proteins in the distal convolution, the distinct differences in the topology of transporters along the distal convolution limit direct extrapolation of data from one species to the other. Discrepancies between the urinary excretion pattern of humans suffering from a defined gene defect for a given transport protein and genetically engineered mouse models with the respective defect might lie, in part, in the different species-speciﬁc organizations of the distal convolution and of the kidney.

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