Regulated expression of pendrin in rat kidney in response to chronic NH$_4$Cl or NaHCO$_3$ loading

SEBASTIAN FRISCHE,1,2 TAE-HWAN KWON,1,3 JØRGEN FRØKIÆR,1,4
KIRSTEN M. MADSEN,5 AND SØREN NIELSEN1,2

1The Water and Salt Research Center, 2Institute of Anatomy, and 4Institute of Experimental Clinical Research, University of Aarhus, DK-8000 Aarhus C, Denmark; 3Department of Physiology, School of Medicine, Dongguk University, 780-714 Kyungju, Korea; and 5Department of Medicine, University of Florida, Gainesville, Florida

Submitted 15 July 2002; accepted in final form 10 October 2002

Frische, Sebastian, Tae-Hwan Kwon, Jørgen Frøkiær, Kirsten M. Madsen, and Søren Nielsen. Regulated expression of pendrin in rat kidney in response to chronic NH$_4$Cl or NaHCO$_3$ loading. Am J Physiol Renal Physiol 284: F584–F593, 2003. First published October 22, 2002; 10.1152/ajprenal.00254.2002.—The anion exchanger pendrin is present in the apical plasma membrane of type B and non-A-non-B intercalated cells of the cortical collecting duct (CCD) and connecting tubule and is involved in HCO$_3$ secretion. In this study, we investigated whether the abundance and subcellular localization of pendrin are regulated in response to experimental metabolic acidosis and alkalosis with maintained water and sodium intake. NH$_4$Cl loading (0.033 mmol NH$_4$Cl/g body wt for 7 days) dramatically reduced pendrin abundance to 22 ± 4% of control values (n = 6, P < 0.005). Immunoperoxidase labeling for pendrin showed reduced intensity in NH$_4$Cl-loaded animals compared with control animals. Moreover, double-label laser confocal microscopy revealed a reduction in the fraction of cells in the CCD exhibiting pendrin labeling to 65% of the control value (n = 6, P < 0.005). Conversely, NaHCO$_3$ loading (0.033 mmol NaHCO$_3$/g body wt for 7 days) induced a significant increase in pendrin expression to 153 ± 11% of control values (n = 6, P < 0.01) with no change in the fraction of cells expressing pendrin.

Immunoelectron microscopy revealed no major changes in the subapical distribution, with abundant labeling in both the apical plasma membrane and the intracellular vesicles in all conditions. These results indicate that changes in pendrin protein expression play a key role in the well-established regulation of HCO$_3$ secretion in the CCD in response to chronic changes in acid-base balance and suggest that regulation of pendrin expression may be clinically important in the correction of acid-base disturbances.

collecting duct; acid-base balance; intercalated cells; electron microscopy; immunocytochemistry

THE INTERCALATED CELLS OF THE collecting duct play an important role in acid-base regulation in the mammalian kidney. On the basis of both the morphological characteristics (30) and the subcellular localization of acid-base transporters (2, 7, 14), two main types of intercalated cells, type A and type B, can be distinguished in the cortical collecting duct (CCD) and connecting tubule (CNT).

Type A intercalated cells secrete protons into the urine and reabsorb HCO$_3$ to the blood (23). They exhibit the vacuolar proton pump in the apical plasma membrane and apical vesicles and the kidney splice variant of anion exchanger 1 (AE1) in the basolateral plasma membrane (2). Type B intercalated cells operate in the reverse mode. They secrete HCO$_3$ in exchange for Cl$^-$ across the apical membrane (17, 23), whereas protons are secreted to the systemic circulation by the vacuolar type H$^+$-ATPase localized in the basolateral membrane. Recent studies have demonstrated that the anion exchanger pendrin is present in the apical domain of type B intercalated cells in the CCD of both rat and mouse kidney (19). Moreover, a comparison between pendrin-deficient and wild-type mice revealed that pendrin is essential for HCO$_3$ secretion in the CCD after NaHCO$_3$ loading (19). Pendrin has also been shown to be expressed in human kidney (15).

A third type of intercalated cell, non-A-non-B (12), is present in low numbers in the CCD and CNT of rats but is abundant in mouse CNT (10). The non-A-non-B type intercalated cells exhibit vacuolar proton pumps as well as pendrin (10, 13) in the apical plasma membrane and no basolateral AE1 (10). The function of non-A-non-B type intercalated cells has not been investigated; accordingly, the role of pendrin in these cells remains unclear.

The rat CCD is capable of either net HCO$_3$ secretion or net HCO$_3$ absorption, depending on the systemic acid-base status (4). CCD segments from 24-h NH$_4$Cl-loaded rats absorb HCO$_3$, and CCD from 24-h NaHCO$_3$-loaded rats secrete HCO$_3$ (4). In CCD from rats made acutely alkalotic by deoxyxorticosterone injections (16) or peritoneal dialysis against an NaHCO$_3$ solution (8), HCO$_3$ secretion is increased and is dependent on luminal Cl$^-$. Furthermore, CCD from fasted rats absorb HCO$_3$, but this is reversed to secretion if

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
the CCD are perfused with 100 mM Cl\(^-\) (16). Thus HCO\(_3^\)\(^-\) secretion in the rat CCD is regulated in response to changes in systemic acid-base status. However, the molecular and cellular/subcellular events that are responsible for the functional regulation of HCO\(_3^\)\(^-\) secretion remain to be established.

Previous studies have described the cellular response to acid-base disturbances in the rat CCD. In type A intercalated cells, the apical membrane area is increased in response to respiratory acidosis (30). This has been interpreted as the result of vesicle trafficking to the apical plasma membrane increasing the number of active proton pumps in the apical plasma membrane in response to acidosis (6). In contrast, acute metabolic alkalosis results in a reduced apical membrane area of type A intercalated cells in rat CCD and reduced density of H\(^+\)-ATPase in the apical plasma membrane (29).

These and other observations (6) suggest that acute fi- 

cking of H\(^+\)-ATPase between the apical plasma membrane and intracellular tubular vesicles and changes in AE1 synthesis (9, 20, 27).

Membrane Fractionation and Immunoblotting

Kidneys from 6 rats from each experimental group were used. Tissue from the cortex/outer stripe of the outer medulla was homogenized in 0.3 M sucrose, 25 mM imidazole, and 1 mM EDTA, pH 7.2, containing 8.5 μM leupeptin and 1 mM phenylmethylyl sulfonfonyfluoride, by using an ultraturrax T8 homogenizer (IKA Labortechnik) at maximum speed for 30 s, and the homogenates were centrifuged in an Eppendorf centrifuge at 4,000 g for 15 min at 4°C to remove whole cells, nuclei, and mitochondria. The supernatant was then centrifuged at 200,000 g for 1 h to produce a pellet containing membrane fractions enriched for both plasma membranes and intracellular vesicles. The samples were prepared for gel electrophoresis by adding Laemmli sample buffer containing 2% SDS (final concentration) to the resuspended pellets.

Antibodies

Polyclonal antibodies raised against a synthetic peptide corresponding to 22 amino acids, MEAENAEELDVQDEAMRRSLAS, of the COOH terminal of mouse pendrin were used to identify pendrin as previously described (13). A monoclonal antibody against rat Calbindin (RD1-CALBIND-abm, Research Diagnostics) was used to distinguish CNTs from collecting ducts in sections of paraffin-embedded rat kidney.

Electrophoresis and Immunoblotting

Samples of rat kidney membranes (see Membrane Fractionation and Immunoblotting for details) were loaded on 9% polyacrylamide minigels (Bio-Rad) and run for 1.5 h at 130 V. After transfer by electroelution (100 V, 1 h) to nitrocellulose membranes, blots were blocked with 5% milk in PBS-T (80 mM Na\(_2\)HPO\(_4\), 20 mM NaH\(_2\)PO\(_4\), 100 mM NaCl, and 0.1% Tween 20, pH 7.5) for 1 h, and incubated overnight at 4°C with anti-pendrin antibodies. The labeling
was visualized with horseradish peroxidase-conjugated secondary antibodies (diluted 1:3,000; P448, DAKO, Glostrup, Denmark) by using an enhanced chemiluminescence system (Amersham International). The chemiluminescence was recorded on film, which was subsequently scanned with a flatbed scanner. Densitometry was performed by using a custom-made computer program, Easy-Gel (David Marples, University of Leeds, Leeds, UK, unpublished).

**Immunohistochemistry**

Kidneys from 6 rats from each experimental group were fixed by retrograde perfusion via the aorta with 4% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.4, and postfixed for 2 h in the same fixative. Kidney slices containing all kidney zones were dehydrated and embedded in paraffin. The paraffin-embedded tissues were cut at 2 μm on a rotary microtome (Leica, Heidelberg, Germany). The sections were dewaxed and rehydrated. To reveal antigens, sections were placed in 1 mM Tris buffer (pH 9.0) supplemented with 0.5 mM EGTA and heated in a microwave oven for 10 min. Nonspecific binding of Ig was prevented by incubating the sections in 50 mM NH4Cl for 30 min followed by blocking in PBS supplemented with 1% BSA, 0.05% saponin, and 0.2% gelatin. Sections were incubated overnight at 4°C with pendrin antibodies diluted in 10 mM PBS, pH 7.4, containing 0.1% Triton X-100 and 0.1% BSA. For light microscopy, sections were incubated with horseradish peroxidase-linked goat anti-rabbit secondary antibodies (P448, DAKO, Glostrup, Denmark), labeling was visualized by diaminobenzidine technique, and the sections were counterstained with Mayer’s hematoxylin. For laser confocal microscopy, calbindin was localized with mouse monoclonal antibodies that were mixed with the antibody against pendrin. The labeling was visualized with an Alexa 488-conjugated goat anti-rabbit antibody (diluted 1:200; Molecular Probes) mixed with an Alexa 488-conjugated goat anti-rabbit antibody (diluted 1:200; Molecular Probes). Confocal laser microscopy was carried out with a Leica SP2 laser confocal microscope.

**Cell Counting**

To evaluate whether the fractions of cells in CNT and CCD showing immunoreactivity for pendrin were changed in acidosis and alkalosis, sections labeled for calbindin and pendrin were analyzed as follows. First, cross sections of CNT and pendrin, and cross sections of CCD were identified by calbindin labeling for both calbindin and pendrin, and cross sections of CCD were identified as tubules with labeling for both calbindin and pendrin, and cross sections of CCD were identified as tubules with labeling for both calbindin and pendrin. The labeling was visualized with a differential interference contrast (DIC) image obtained concomitantly with the fluorescence images. Third, the nuclei pertaining to cells that also labeled for pendrin were counted. One kidney section from each of five NH4Cl-loaded and five control rats and from each of four NaHCO3-loaded and three control rats was inspected. In each section, at least five cross sections of CNT and five cross sections of CCD were identified, and at least 62 cells were counted from each tubule segment in each animal. In total 1,943 cells were counted. The fraction of pendrin-labeled cells was calculated as the number of nuclei in pendrin-positive cells found in one animal divided by the total number of nuclei counted in this animal. This procedure underestimates the total number of cells in CNT and CCD, because tubular cross sections devoid of pendrin labeling were not counted. Therefore, the absolute fraction of pendrin-positive cells is overestimated. However, the measurements are only intended for comparison within this study, enabling a semiquantitative interpretation of the labeling patterns between treated and control rats.

Before averaging, normalization with respect to control values and further statistical analysis, the fraction scale data were arc-sin transformed to obtain normality (34).

**Immunoelectron Microscopy**

For immunoelectron microscopy, small pieces of kidney cortex were cut from slices of fixed kidney (see Immunohistochemistry), cryoprotected in 2.3 M sucrose, and frozen in liquid nitrogen. The frozen samples were freeze-substituted in a Reichert AFS freeze substitution unit. In brief, the samples were sequentially equilibrated over 3 days in methanol containing 0.5% uranyl acetate at temperatures gradually raised from −80 to −70°C, rinsed in pure methanol for 24 h while increasing the temperature from −70 to −45°C, and infiltrated with and graded Lowicryl HM20 and methanol solutions (1:1, 2:1) and pure Lowicryl HM20 before UV polymerization for 2 days at −45°C and 2 days at 0°C. Immunolabeling was performed on ultrathin Lowicryl HM20 sections. Sections were pretreated with the saturated solution of NaOH in absolute ethanol (2–3 s), rinsed, and preincubated for 10 min with 0.1% sodium borohydride and 50 mM glycine in 0.05 M Tris, pH 7.4, containing 0.1% Triton-X 100. Sections were rinsed and incubated overnight at 4°C with primary antibodies diluted in 0.05 M Tris, pH 7.4, containing 0.1% Triton-X 100 with 0.2% milk (diluted 1:200). After being rinsed, sections were incubated for 1 h at room temperature with goat anti-rabbit IgG conjugated to 10-nm colloidal gold particles (1:50; GAR.EM10, BioCell Research Laboratories, Cardiff, UK). The sections were stained with uranyl acetate and lead citrate before examination in a Philips Morgagni electron microscope operating at 70 kV.

**RESULTS**

**NH4Cl-Loaded Rats Showed Reduced Urine pH, Decreased Plasma Sodium, Increased Plasma Potassium, and Increased Urine Osmolality**

The urine and blood acid-base parameters of the NH4Cl-loaded and control groups are shown in Table 1. Urine proton concentration differed significantly between the experimental group and the control group; accordingly, marked differences in urine pH were seen (5.76 vs. 7.94). Blood acid-base parameters (plasma [H+] and plasma [HCO3−], where brackets indicate concentration, plasma total CO2, and plasma PCO2) were unchanged between experimental and control groups.

**Pendrin Abundance in NH4Cl-Loaded Rats Was Markedly Reduced**

Semiquantitative immunoblotting of 4,000-g supernatants of homogenized rat kidney cortex and outer stripe of the outer medulla from NH4Cl-loaded rats showed a marked reduction in the amount of detectable pendrin: 22 ± 4 vs. 100 ± 11%, P < 0.005 (Fig. 1, A and B). Similarly, immunoperoxidase labeling for pendrin in sections of paraffin-embedded kidneys from NH4Cl-loaded rats showed less intense staining in the outer cortex (CNT and CCD segments) than sections from control rats when analyzed at low magnification (Fig. 2, A and B). Kidney sections from 6 NH4Cl-loaded...
Table 1. Blood and urine data

<table>
<thead>
<tr>
<th></th>
<th>NH₄Cl Loaded</th>
<th>NH₄Cl Control</th>
<th>NaHCO₃ Loaded</th>
<th>NaHCO₃ Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (urine pH and [H⁺] data)</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Urinary pH</td>
<td>5.76</td>
<td>7.94</td>
<td>8.77</td>
<td>7.40</td>
</tr>
<tr>
<td>Urinary [H⁺]·10⁻⁶ M</td>
<td>175 ± 121.7*</td>
<td>1.14 ± 1.005*</td>
<td>0.017 ± 0.0033*</td>
<td>3.99 ± 1.40</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Plasma pH</td>
<td>7.40</td>
<td>7.38</td>
<td>7.38</td>
<td>7.38</td>
</tr>
<tr>
<td>Plasma [H⁺]·10⁻⁶ M</td>
<td>4.02 ± 0.304*</td>
<td>4.16 ± 0.211*</td>
<td>4.12 ± 0.222*</td>
<td>4.15 ± 0.207*</td>
</tr>
<tr>
<td>Plasma HCO₃⁻, mM</td>
<td>29 ± 1.8</td>
<td>27 ± 1.0</td>
<td>28 ± 1.2</td>
<td>29 ± 1.0</td>
</tr>
<tr>
<td>Plasma total CO₂, mM</td>
<td>30 ± 1.8</td>
<td>29 ± 1.0</td>
<td>29 ± 1.2</td>
<td>30 ± 1.0</td>
</tr>
<tr>
<td>Plasma Pco₂, kPa</td>
<td>6.3 ± 0.34</td>
<td>6.2 ± 0.17</td>
<td>6.3 ± 0.42</td>
<td>6.5 ± 0.34</td>
</tr>
<tr>
<td>Plasma Na, mM</td>
<td>135.5 ± 1.22*</td>
<td>138.5 ± 1.76*</td>
<td>136.7 ± 0.82</td>
<td>134.2 ± 2.48 *</td>
</tr>
<tr>
<td>Plasma K, mM</td>
<td>5.4 ± 0.36*</td>
<td>4.7 ± 0.19</td>
<td>4.6 ± 0.42</td>
<td>4.7 ± 0.34</td>
</tr>
<tr>
<td>Plasma creatinine, μM</td>
<td>31.5 ± 2.59</td>
<td>28.3 ± 2.50</td>
<td>28.7 ± 4.84</td>
<td>27.3 ± 1.63</td>
</tr>
<tr>
<td>Urine osmolality, mosmol/kgH₂O</td>
<td>1,263 ± 177*</td>
<td>864 ± 111</td>
<td>1,253 ± 52</td>
<td>1,280 ± 106 *</td>
</tr>
<tr>
<td>Plasma osmolality, mosmol/kgH₂O</td>
<td>301 ± 2</td>
<td>304 ± 4</td>
<td>300 ± 3</td>
<td>300 ± 3</td>
</tr>
<tr>
<td>(U/P)osm</td>
<td>4.2 ± 0.65*</td>
<td>2.8 ± 0.38</td>
<td>4.2 ± 0.17</td>
<td>4.3 ± 0.38</td>
</tr>
</tbody>
</table>

Values are means ± SD where applicable. n, No. of rats; [H⁺], proton concentration; Pco₂, partial pressure of CO₂; Ccr, creatinine clearance; BW, body weight; (U/P)osm, urine-to-plasma osmolality ratio. *P < 0.05, (Student’s t-test); mean value of treated rats compared with mean value of corresponding control rats.

rats and 5 control rats were inspected and showed patterns consistent with the examples shown.

Fraction of CDD and CNT Cells with Pendrin Immunoreactivity Was Reduced in NH₄Cl-Loaded Rats

At higher magnification, the reduced labeling of pendrin was also apparent, although the difference in the intensity of labeling at the level of individual CCD and CNT profiles and intercalated cells was less pronounced (Fig. 2, C and D). Both CNT and CCD segments showed reduced labeling. Furthermore, it appeared that the number of cells exhibiting pendrin labeling was markedly reduced in response to NH₄Cl loading (Fig. 2, C and D). To examine this further, laser confocal and DIC microscopy were performed by using double-immunolabeled (for pendrin and calbindin) sections of paraffin-embedded kidneys. To evaluate whether the fraction of cells with detectable pendrin immunoreactivity was changed, pendrin-labeled cells and the total number of cells in cross-sectioned tubules with pendrin-labeled cells in CNT and CDD were counted. To illustrate the counting procedure, an example of the images used for cell counting is shown in Fig. 3, A–D. The results revealed that the fraction of cells exhibiting pendrin immunoreactivity in CDD was significantly reduced in NH₄Cl-loaded rats compared with control values (65 ± 4% of the control value, P < 0.005; Table 2; Fig. 3E). In CNT, the fraction of cells exhibiting pendrin immunoreactivity was not significantly different from the control value (87 ± 5% of control value, P = 0.26; Table 2; Fig. 3).

Subcellular Localization of Pendrin Was Not Changed in Response to Chronic NH₄Cl Loading

Immunoelectron microscopical analysis of pendrin localization confirmed reduced immunogold labeling in NH₄Cl-loaded rats compared with control animals. However, there were no apparent differences in the subcellular localization of pendrin between NH₄Cl-loaded and control rats (Fig. 4, A and B). In both groups, pendrin was localized at the apical plasma membrane and in intracellular vesicular structures in the apical part of type B intercalated cells.

NaHCO₃-Loaded Rats Showed Increased Urine pH

The urine and blood acid-base parameters of the NaHCO₃-loaded and control groups are shown in Table 1. Urine proton concentration differed significantly
between the experimental group and the control group; accordingly, marked differences in urine pH were seen (8.77 vs. 7.40). Blood acid-base parameters (plasma $[H^+]$, plasma $[HCO_3^-]$, plasma total CO$_2$, and plasma PCO$_2$) were unchanged between the NaHCO$_3$-loaded and control group.

**Pendrin Abundance in NaHCO$_3$-Loaded Rats Was Markedly Increased**

Semiquantitative immunoblotting of 4,000-g supernatants of homogenized rat kidney cortex and outer stripe of the outer medulla from NaHCO$_3$-loaded rats showed a significant increase in the amount of detectable pendrin: 153 ± 11 vs. 100 ± 12%, $P < 0.01$ (Fig. 5, A and B). Consistent with this, immunoperoxidase-labeled sections of paraffin-embedded kidneys from NaHCO$_3$-loaded rats (Fig. 6, A and C) exhibited an increase in the intensity of pendrin immunostaining compared with sections from control rats (Fig. 6, B and D). The increase in labeling intensity was equally distributed over the labeled cells, i.e., the change was not only observed in a subset of cells. Kidney sections from four NaHCO$_3$-loaded rats and three NaHCO$_3$-control rats were inspected.

**Fraction of CCD and CNT Cells with Pendrin Immunoreactivity Was Unchanged in NaHCO$_3$-Loaded Rats**

Laser confocal microscopy and DIC microscopy of double-immunolabeled (for pendrin and calbindin) paraffin sections revealed that the fraction of pendrin-labeled epithelial cells in CNT and CCD was unchanged in NaHCO$_3$-loaded rats (CNT: 102 ± 4% of control values, $P = 0.80$; CCD: 112 ± 9% of control values, $P = 0.41$; Table 2; Fig. 7).

**Subcellular Localization of Pendrin Was Not Changed in Response to NaHCO$_3$ Loading**

Electron microscopical investigation of immunolabeled kidney sections from NaHCO$_3$-loaded rats (Fig. 8A) did not show consistent differences compared with control animals (Fig. 8B) regarding the subcellular localization of pendrin. In both groups, pendrin was localized at the apical plasma membrane and in intracellular vesicular structures in the apical part of type B intercalated cells. The relative distribution of labeling between the plasma membrane and the intracellular compartment varied from cell to cell in both groups,
but no quantitative measures of this variation were obtained.

**DISCUSSION**

This study documents a marked reduction in pendrin abundance in the CCD and CNT of rats in response to chronic NH₄Cl loading. In contrast, chronic NaHCO₃ loading resulted in a significant increase in pendrin abundance. These findings are in accordance with results of previous studies (4, 8, 16, 33) of HCO₃⁻/H₂CO₃ transport in the CCD and strongly indicate that the regulation of HCO₃⁻ secretion in the rat CCD in response to acid-base disturbances involves changes in pendrin abundance in the type B intercalated cells. The results also provide additional support for the importance of intercalated cells in the CCD and CNT in the regulation of systemic acid-base balance. On the basis of these observations, we conclude that HCO₃⁻/H₂CO₃ secretion can be stimulated or inhibited through changes in pendrin abundance in intercalated cells in the CCD and CNT. Thus the regulation of pendrin expression in the collecting duct represents a molecular mechanism to control HCO₃⁻ secretion and correct acid-base disturbances.

**Pendrin Abundance Is Increased in NaHCO₃-Loaded Rats and Decreased in NH₄Cl-Loaded Rats**

Because the increase in pendrin after NaHCO₃ loading is not restricted to a subset of cells, it is most likely due to an increased amount of pendrin in both intercalated type B cells and intercalated cells of the non-A-non-B type. Similarly, the reduction in pendrin seen by immunoblotting after NH₄Cl loading cannot solely be due to changes in non-A-non-B cells because of the low fraction of these cells in rat CNT and CCD (5.9 and 2.1%, respectively) (10).

The changes in pendrin abundance demonstrated in this study are in agreement with the results of physiological studies indicating an increased capacity for HCO₃⁻ secretion in CCD from NaHCO₃-loaded animals and a reduced capacity for HCO₃⁻ secretion in NH₄Cl-loaded animals (4, 16).

The mechanism of HCO₃⁻ secretion in CCD has been extensively studied. Star et al. (28) found that replacement of luminal and peritubular Cl⁻ with
Glucuronate decreases HCO\textsubscript{3}\textsuperscript{-} secretion significantly, indicating that HCO\textsubscript{3}\textsuperscript{-} secretion in CCD is dependent on Cl\textsuperscript{-}. Subsequent studies by several laboratories have confirmed that HCO\textsubscript{3}\textsuperscript{-} secretion in the CCD of both rats and rabbits is mediated by apical Cl\textsuperscript{-}/HCO\textsubscript{3}\textsuperscript{-} exchange (23). Recent studies have demonstrated that the anion exchanger pendrin is localized at the apical domains of HCO\textsubscript{3}\textsuperscript{-}-secreting type B intercalated cells, and elegant studies using transgenic mice lacking pendrin showed that pendrin is involved in HCO\textsubscript{3}\textsuperscript{-} secretion (19). Subsequently, pendrin was shown also to be present in type non-A-non-B intercalated cells (13). The present study indicates that regulation of HCO\textsubscript{3}\textsuperscript{-} secretion in CCD is, at least in part, dependent on changes in pendrin abundance in intercalated cells.

The majority of filtered HCO\textsubscript{3}\textsuperscript{-} is reabsorbed by the proximal tubule. Consistent with this, high rates of urinary HCO\textsubscript{3}\textsuperscript{-} excretion during alkali loading or in response to metabolic alkalosis have largely been attributed to inhibition of HCO\textsubscript{3}\textsuperscript{-} reabsorption in the proximal tubule (3). However, the final regulation of urine acidification takes place in the collecting duct, which is a site of both reabsorption and secretion of HCO\textsubscript{3}\textsuperscript{-}. On the basis of the results of this study, we propose that the fine control of urinary HCO\textsubscript{3}\textsuperscript{-} excretion is achieved by regulation of HCO\textsubscript{3}\textsuperscript{-} secretion through changes in pendrin abundance in the CNT and CCD. Thus when changes in HCO\textsubscript{3}\textsuperscript{-} transport are required to achieve acid-base homeostasis, for example, in subchronic or chronic metabolic acidosis or alkalosis, HCO\textsubscript{3}\textsuperscript{-} secretion can be inhibited or stimulated through the regulation of pendrin expression in the intercalated cells.

This mechanism may explain the results of a study of the effect of NaCl infusion on the correction of alkalosis induced by HCO\textsubscript{3}\textsuperscript{-} loading. Here, it was found that the increase in urinary HCO\textsubscript{3}\textsuperscript{-} secretion after NaCl infusion was much higher in alkalotic rats than in control rats (33). We propose that this may be related to an increased abundance of pendrin in HCO\textsubscript{3}\textsuperscript{-}-loaded rats, as documented in the present study.

The regulatory mechanisms responsible for the changes in pendrin abundance are as yet unclear; however, a cAMP-dependent intracellular pathway is possibly involved, as indicated by the finding that cAMP increases rabbit CCD HCO\textsubscript{3}\textsuperscript{-} secretion (21). It has been proposed that the prostacyclin-induced increase in distal tubule HCO\textsubscript{3}\textsuperscript{-} secretion acts through this pathway (31). Furthermore, a recent study has indicated a possible role of endothelin-1 in regulating increased distal tubular acidification in response to acid ingestion (32). Hypothetically, endothelin-1 could increase tubular acidification by reducing pendrin abundance in CCD type B intercalated cells.
as found in NH₄Cl-loaded animals in the present study.

**Fraction of CCD and CNT Cells Showing Pendrin Immunoreactivity Was Reduced in NH₄Cl-Loaded Rats, Whereas It Was Unchanged in NaHCO₃-Loaded Rats**

Controversy exists about whether the relative numbers of type A and type B cells change during systemic acidosis (23). On the basis of in vitro studies in the CCD and in cultured collecting duct cells, it has been proposed that a reversal of polarity of type B intercalated cells might account for the changes in H⁺-ATPase labeling patterns observed in various acid-base disturbances (1, 25, 26). However, the presence of distinct anion exchangers in type A and type B intercalated cells excludes a simple relocation of the intercalated cell proteins as the mechanism behind the observed changes. On the other hand, it is possible that a more extensive epithelial remodeling might lead to changes in the number of cells expressing pendrin and thus explain the changes in the abundance of pendrin. To determine whether changes in acid-base status affect the fraction of pendrin-positive cells, we compared estimates of the fractions of pendrin-labeled cells in CNT and CCD of NH₄Cl-loaded, NaHCO₃-loaded, and control animals.

As shown in Fig. 3E, the reduced amount of pendrin in the kidney cortex of NH₄Cl-loaded rats was accompanied by a significant reduction in the fraction of cells in the CCD that label for pendrin to 65% of control values. The reduction to 87% of control values seen in CNT was not statistically significant but indicates that intercalated cells in the CNT may also lose pendrin immunoreactivity in response to...
NH4Cl loading, although to a lesser extent than that in the CCD.

Pendrin immunoreactivity was never observed in the basolateral domain, so these results do not provide any evidence for a polarity change, i.e., relocation of the intercalated cell proteins. There are several possible explanations for the observed decrease in the fraction of pendrin-positive cells in the CCD and CNT: 1) a decrease in pendrin abundance in individual cells leading to levels of expression undetectable by immunohistochemistry; 2) a disappearance of pendrin-expressing cells, e.g., by apoptosis or other forms of cell deletion; and 3) a transformation of pendrin-expressing cells into other cell types, a scenario that would provide support for the remodeling hypothesis previously proposed for type B intercalated cells. Distinguishing these three possible explanations for the reduced fraction of CCD and CNT cells exhibiting pendrin immunoreactivity is outside the scope of the present study and awaits future investigations.

It should be noted that support for the possibility of epithelial remodeling was provided in a recent study reporting a decrease in the percentage of type B intercalated cells in the CCD of rats after chronic treatment with acetazolamide, a carbonic anhydrase inhibitor, for 14 days (5). This decrease was associated with an increase in the percentage of type A intercalated cells not only in the CCD but also in the collecting duct in the inner stripe of the outer medulla.

Recent evidence from isolated perfused rabbit CCD indicates that individual type B intercalated cells lose apical Cl-/HCO3- exchange activity after 3 h incubation at pH 6.8 (24). This suggests the presence of a rapid mechanism for regulation of HCO3- secretion in type B intercalated cells consistent with previous experiments in isolated perfused CCD from rats (8, 16) and rabbits (21). The disappearance of apical Cl-/HCO3- exchange activity was accompanied by a reduction in apical plasma membrane area, which is indicative of endocytotic internalization of Cl-/HCO3- exchangers (26), as previously proposed (8). We believe that irrespective of the cellular mechanisms, the previously observed reduction in the capacity for HCO3- secretion by rat CCD in response to chronic NH4Cl loading is most likely due to a reduction in the abundance of active pendrin anion exchangers.

Interestingly, there were no significant changes in the fractions of CCD and CNT cells showing pendrin immunoreactivity in response to NaHCO3 loading. These observations suggest that the previously demonstrated increase in HCO3- secretion during various alkalotic conditions is likely to be mediated by an increase in pendrin expression/activity in already existing type B intercalated cells rather than being associated with changes in the number of HCO3- secreting cells.

**Subcellular Localization of Pendrin Is Unchanged in NH4Cl-Loaded and NaHCO3-Loaded Rats Compared with Control Rats**

The observed changes in the levels of expression of pendrin were not accompanied by qualitative changes in the subcellular localization of pendrin within the B-cells and non-A-non-B cells, as determined by immunoelectron microscopy. Pendrin was found in the apical plasma membrane and in intracellular vesicular structures in the apical part of intercalated type B and non-A-non-B cells in NH4Cl-loaded, NaHCO3-loaded, and two control groups. Thus there was no evidence that dramatic changes in trafficking or apical sorting of pendrin might be responsible for the regulation of CCD HCO3- secretion in response to chronic acid-base disturbances. Because the animals in this study were subject to chronic acid and base loading, the subcellular localization of pendrin most likely represents steady-state situations in animals, which have adapted to the constant load. This is supported by the observation that plasma pH and total CO2 levels were similar in all of the experimental groups. Thus the present results do not preclude the occurrence of transient short-term regulation involving trafficking of pendrin molecules among cellular compartments in response to the acute disturbances. Rather, it is quite possible that short-term acid-base disturbances may induce rapid changes in the subcellular localization of pendrin, which might explain the documented reduction in cAMP-induced HCO3- secretion by rabbit CCD during...
NH₄Cl loading (21). The purpose of this study was to evaluate the effect of chronic acid-base disturbances on pendrin expression and localization; thus features pertaining to short-term regulation may have passed unnoticed. Whether insertion and removal of pendrin molecules in the apical plasma membrane are responsible for rapid adjustments of HCO₃⁻ secretion in CCD should be pursued in future studies.

In conclusion, the present study demonstrates that pendrin protein expression in the kidney is tightly regulated in response to acid-base disturbances and suggests that HCO₃⁻ secretion in the CCD and CNT is regulated through changes in pendrin abundance. Thus when changes in collecting duct HCO₃⁻ transport are required to achieve acid-base homeostasis, for example, in metabolic acidosis or alkalosis, HCO₃⁻ secretion can be inhibited or stimulated through the regulation of pendrin abundance in the kidney.

The authors thank Mette F. Vistisen, Lotte Vallentin Holbech, Helle Hayer, Zhila Nikrozi, Gitte Christensen, Merete Pedersen, and Inger Merete Paulsen for technical assistance. We thank the reviewers for their comments.

The Water and Salt Research Center at the University of Aarhus is established and supported by the Danish National Research Foundation (Danmarks Grundforskningsfond). Support for this study was additionally provided by The European Commission (Contract number QLKI-CT-2000-0078).

REFERENCES