Similar chloride channels in the connecting tubule and cortical collecting duct of the mouse kidney

Antoine Nissant, Marc Paulais, Sahran Lachheb, Stéphane Lourdel, and Jacques Teulon

Institut des Cordeliers, Laboratoire de Physiologie et Génomique des Cellules Rénales, UMR 7134, Université Pierre et Marie Curie and Centre National de la Recherche Scientifique, Paris, France

Submitted 4 July 2005; accepted in final form 9 January 2006

Nissant, Antoine, Marc Paulais, Sahran Lachheb, Stéphane Lourdel, and Jacques Teulon. Similar chloride channels in the connecting tubule and cortical collecting duct of the mouse kidney. Am J Physiol Renal Physiol 290: F1421–F1429, 2006. First published January 10, 2006; doi:10.1152/ajprenal.00274.2005.—Using the patch-clamp technique, we investigated Cl− channels on the basolateral membrane of the connecting tubule (CNT) and cortical collecting duct (CCD). We found a ~10-pS channel in CNT cell-attached patches. Substitution of sodium gluconate for NaCl in the pipette shifted the reversal potential by +25 mV, whereas N-methyl-D-glucamine chloride had no effect, indicating anion selectivity. On inside-out patches, we determined a selectivity sequence of Cl− > Br− > NO3− > F−, which is compatible with that of ClC-K2, a Cl− channel in the distal nephron. In addition, the number of open channels (NPo) measured in cell-attached patches was significantly increased when Ca2+ concentration or pH in the pipette was increased, which is another characteristic of ClC-K. These findings suggest that the basis for this channel is ClC-K2. A similar Cl− channel was found in CCD patches. Because CNT and CCD are heterogeneous tissues, we studied the cellular distribution of the Cl− channel using recording conditions (KCl-rich solution in the pipette) that allowed us to detect simultaneously Cl− channels and inwardly rectifying K+ channels. We detected Cl− channels alone in 45% and 42% and K+ channels alone in 51% and 58% of CNT and CCD patches, respectively. Cl− and K+ channels were recorded simultaneously from two patches (4% of patches) in the CNT and from none of the patches in the CCD. This indicates that Cl− and K+ channels are located in different cell types, which we suggest may be the intercalated cells and principal cells, respectively.

ClC-K; renal tubule; intercalated cell

The purpose of this study was to investigate Cl− channels in the mouse connecting tubule (CNT) and cortical collecting duct (CCD), two heterogeneous segments composed of principal and intercalated cells. The CCD is involved in the fine regulation of ion, water, and acid-base balance, the principal cells notably participating in Na+ absorption via the apically located epithelial Na+ channel and basolateral Na+-K+-ATPase. A similar role for the CNT has emerged only recently from electrophysiology and immunofluorescence studies (5, 21). Rubera et al. (32) demonstrated an intact Na+ balance in mice in which the functional epithelial Na+ channel was missing from the CCD. In parallel to Na+ absorption, there is evidence that Cl− is absorbed in the CNT and CCD via paracellular and transcellular routes. Transcellular absorption involves intercalated cells, implying that the basolateral membrane must be permeable to Cl− (19, 38, 39). On the basis of the apical/basolateral location of the Cl−/HCO3− exchanger and H+−ATPase, the present classification distinguishes three types of intercalated cells: type A (basolateral Cl−/HCO3− exchanger and apical H+−ATPase), type B (apical Cl−/HCO3− exchanger and basolateral H+−ATPase), and non-A non-B (apical Cl−/HCO3− exchanger and H+−ATPase). Electrophysiological studies, mostly conducted on rabbit CCD and CNT, have defined the conductance properties of type A and type B intercalated cells with the use of the in vitro microperfusion technique and membrane potential recording using microelectrodes (13, 20, 26, 34). These studies attribute major conductance to Cl− and comparatively low conductance to K+ in the basolateral membranes of the intercalated cells. Thus Cl− conductance could be in a position to contribute to the net Cl− absorption in type B (and probably non-A non-B) intercalated cells, where the Cl−/HCO3− exchanger pendrin is located on the apical membrane (17, 31, 42). Non-A non-B intercalated cells, which have not been reported in the rabbit, represent the major intercalated cell type in the mouse CNT (16).

We know little about the Cl− channels in these parts of the renal tubule: no patch-clamp analysis of Cl− channels in the CNT is available, and, to our knowledge, the only report concerning native CCD depicts a double-barreled Cl− channel with a conductance of 45 pS in the basolateral membrane of principal cells of the rabbit CCD (33). Here we report a 10-pS Cl− channel that has properties similar to a Cl− channel in the distal convoluted tubule (DCT) (23) and is probably based on ClC-K2. This channel is located mainly in intercalated cells.

METHODS

Isolation of renal tubules. The experimental protocols were approved by the Veterinary Department of the French Ministry of Agriculture (license no. 7427). Male CD1 mice (15–20 g body wt; Charles River, L’Arbresle, France) were killed by cervical dislocation. The left kidney was perfused with L-15 Leibovitz medium (Sigma, Saint Quentin Fallavier, France) supplemented with collagenase (CLS II, Worthington; 300 U/ml) and removed. Small pieces of cortex were incubated at 37°C for 45–60 min in the same collagenase-containing medium, rinsed, and kept at 4°C (25).

The CNT joins one DCT to one CCD in superficial nephrons and forms a branching structure, known as the arcade, deeper in the kidney and, thus, joins several DCTs to one CCD (14). Most CNTs in this study have been isolated from arcades. Tubular fragments were dissected out under a stereomicroscope just before use and transferred to a petri dish placed on the stage of an inverted microscope (Axiovert 25, Zeiss) for patch clamping. Cell types were distinguished by visual examination. Principal cells, which have a polygonal and flat appear-

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.
ance, predominant (~60%) in the CCD (16); intercalated cells are round and protuberant. The CNT exhibits the same characteristics but contains more ambiguous cells than the CCD.

**Solutions and chemicals.** The tubules were initially bathed in physiological saline (PS) containing (in mM) 140 NaCl, 5 KCl, 1 CaCl2, 1 MgCl2, 10 glucose, and 10 HEPES and adjusted to pH 7.4 with NaOH. Unless otherwise stated, the patch pipettes were filled with a similar solution containing 145 mM NaCl and no KCl. For cell-attached patch recordings, we also used pipette solutions in which 145 mM N-methyl-D-glucamine chloride or 100 mM sodium gluconate was substituted for NaCl. As observed previously for a DCT Cl– channel (23), there was a rapid, irreversible, and total loss of activity when the patches were excised directly into PS (1 mM Ca2+). Thus we routinely excised the patches into a solution containing no Ca2+ (2 mM EGTA) and supplemented with 1 mM ATP, which reduced and considerably slowed channel rundown.

Using a high-K+ (145 mM KCl) solution in the pipette, we performed experiments in which we attempted to record simultaneously Cl– channels (~10-pS conductance) and inwardly rectifying K+ channels (~40-pS inward conductance) in the CNT and CCD. Because the cortical thick ascending limb (CCTAL) basolateral membrane contains K+ and Cl– channels with similar conductance (7, 28), we first verified that these recording conditions did indeed allow us to detect low-conductance Cl– channels in the presence of the higher-conductance K+ channel on this preparation (n = 15; results not shown).

Cl– channel inhibitors and N-ethylmaleimide (NEM) were purchased from Sigma-Aldrich. Diphenylamine-2-carboxylate (DPC), at 0.5 M, and 5-nitro-2-(3-phenylpropylamino)benzoate (NPPB), at 0.1 M, were dissolved in DMSO. DMSO (at the maximum concentration of 0.2% used for 10-3 M DPC) had no effect on channel activity (n = 3). NEM was dissolved in ethanol.

**Current recordings.** The cell-attached and excised, inside-out configurations of the patch-clamp technique (9) were used to record single-channel currents from patches of basolateral membranes. Patch-clamp pipettes were pulled from borosilicate glass (GC150T, List, or model RK 400, Bio-logic), monitored using Axoscope software (Axon Instruments, Foster City, CA), and stored on digital audiotape (DTR-1205, Sony). Potentials across cell-attached and excised membrane patches were corrected for liquid junction potentials as described by Barry and Lynch (1). The liquid junction potentials were measured directly using a procedure described previously (29). Currents carried by anions moving from the outer to the inner face of the patch membrane were considered positive and are shown as upward deflections in the current traces. The experiments were carried out at room temperature (22–27°C).

**Analysis of channel activity.** Single-channel current recordings were filtered at 300 or 500 Hz (low-pass) by an eight-pole Bessel filter (model LPBF-48DG, NPI Electronic, Tamm, Germany) and digitized at a sampling rate of 1–2 kHz with an analog-to-digital converter (Digidata 1200) and Axoscope software. We used pClamp-9 software (Axon Instruments) to measure the open probability (Popen) in inside-out patches from digitized stretches of recording lasting ≥30 s.

In the case of cell-attached patches, which frequently showed a large number of channels, we estimated the number of open channels (Np) according to the following equation: <I–>/<I> = NpECl, where is the unit current amplitude and <I> is the time-averaged Cl– current passing through the patch, with the closed current level as reference. As observed in previous experiments on small-conductance Cl– channels (8, 22, 24), the closed current level was not usually visible because of the high number of channels per patch and the slow kinetics of the channel (22). The closed current level was estimated by inhibiting Cl– channel activity, taking advantage of the fact that this channel is inhibited by an acidic intracellular pH (pH2).

Intracellular compartment, we used Na+–free solution (in which Na+ had been replaced by N-methyl-d-glucamine) to reduce the activity of the Na+/H+ exchanger; in some cases, this solution was supplemented with NEM (0.25 mM), an inhibitor of the H+ pump (25).

Before performing patch-clamp experiments, we used video-enhanced fluorescence microscopy and image processing (Argus 50, Hamamatsu Photonics, Hamamatsu City, Japan) to monitor the induced changes in pH2. Images of 2′,7′-bis-(2-carboxyethyl)-5(6)-carboxy fluorescein-loaded CNT segments were obtained, and ratios were determined and converted to pH2 values as previously described (24).

The control pH2 (7.19 ± 0.01; n = 3) was reduced by 0.38 ± 0.02 (n = 3) after 20 s of exposure to an Na+–free solution (in the absence of NEM) and by 0.51 ± 0.04 (n = 3) after 2 min of exposure to the same solution in the presence of NEM (added 20 s after solution was switched to Na+–free solution). The channels were usually inhibited very efficiently and rapidly; however, the inhibition was not reversible.

Although the slow kinetics of the channel made it possible to identify single-channel openings in patches containing a large number of channels (up to ~30), patches with >40–50 channels generated macroscopic currents. When recorded from cell-attached patches without nonselective cation channels, macroscopic currents should be carried by 10-pS Cl– channels, because K+ channels were not visible under our experimental conditions. We checked the ion selectivity of the macroscopic currents by determining macroscopic current-voltage curves before and after acidification (to determine the zero-current level) under three conditions. The reversal potential (Ere) was close to zero with 145 mM NaCl (0.8 ± 1.7 mV; n = 3) and 145 N-methyl-d-glucamine chloride (~0.7 ± 1.0 mV; n = 4) in the pipette. It shifted to 24.3 ± 0.7 (n = 3) with 45 mM NaCl (100 sodium gluconate) in the pipette, as expected for Cl––selective currents.

**Ion selectivity.** The permeability ratios were calculated by fitting the data points to the current equation of Goldman, Hodgkin, and Katz or, alternatively, when it was not possible to measure the unit currents beyond Ere, by using the voltage form of this equation. For the latter case, individual Ere values were determined from the linear regression of the experimental data points. The Na+–to-Cl– permeability ratio (PNa/Pcl) was estimated using a low-NaCl bath solution containing 14 mM NaCl (succrose added). For anion selectivity, 130 mM NaCl on the bath side was replaced by an equivalent amount of an Na+ salt of the test anion. The activity coefficients used in the calculations were 0.89 for the low-NaCl solution and 0.76 for all other solutions (30).

**Statistics.** Values are means ± SE for the indicated number of measurements (n). Origin software (Microcal, Northampton, MA) was used to fit the data points to equations. Statistical significance was evaluated by Student’s t-test or the Mann-Whitney rank sum test when necessary using SigmaStat software (SPSS, Erkrath, Germany). P < 0.05 was considered significant.

**RESULTS**

**Channel properties in cell-attached patches from the CNT: sensitivity to external pH and Ca2+.** Cell-attached patches formed on the CNT basolateral membranes by use of standard pipette solution (145 NaCl) exhibited two types of channels. One was a cation channel that has been reported elsewhere (2) and was not further investigated. Typical activity of the second channel is shown in Fig. 1A. The current-voltage relationship was linear (Fig. 1B) and reversed at 1.9 ± 4.0 mV; a unit conductance of 10.6 ± 0.9 pS was computed (n = 8). There was no significant change in Ere when N-methyl-d-glucamine chloride (140 mM) was substituted for NaCl in the pipette (~1.1 ± 2.0 mV; n = 8, data not shown), but a large shift to the right was observed in the presence of 100 mM sodium gluconate (plus 45 mM NaCl) in the pipette (25.7 ± 1.2 mV,
The DCT (known modulators of ClC-K (3, 41) and DCT Cl density. A recording with many active Cl excision (mean number of channels per patch mentioned with NEM is shown in Fig. 2. Before inhibition, 10) than 1 mM Ca2+ saline (PS). Dotted lines, closed current level.

Fig. 1. Channel in cell-attached configuration. A: representative current recordings at various clamp potentials (Vc). Bath and pipette contained physiological saline (PS). B: mean single-channel current-voltage (i-Vc) relationship obtained under conditions described in A (n, n = 3–8) or with pipette filled with 100 mM sodium gluconate and 45 mM NaCl (○, n = 3–5). SE is shown as an error bar when it is larger than symbol.

n = 4). This indicates Cl− selectivity (Fig. 1B). The Cl− channel was observed in 78 of 190 patches (41%).

A characteristic feature of the CNT Cl− channel was its high density. A recording with many active Cl− channels before and during the inhibition induced by the Na+ -free solution supplemented with NEM is shown in Fig. 2. Before inhibition, \( N_{P_o} \) was 28.4. Mean \( N_{P_o} \) with 1 mM Ca2+ in the pipette solution (pH 7.4; Fig. 3) was 23 ± 6 (n = 11), which is higher than in the DCT (\( N_{P_o} ~ 8 \) (22) but lower than in the CTAL (\( N_{P_o} ~ 30 \) (24) under comparable conditions of pipette resistance. In a first step toward characterizing the properties of this channel, we investigated the effects of external Ca2+ and pH, which are known modulators of CIC-K (3, 41) and DCT Cl− channels (23, 35). We first compared \( N_{P_o} \) in separate cell-attached patches formed with pipettes containing 1 or 5 mM Ca2+ (Fig. 3). Although \( N_{P_o} \) varied from patch to patch, it was significantly higher in the presence of 5 mM Ca2+ (101 ± 12, n = 10) than 1 mM Ca2+ (23 ± 6, n = 11, \( P < 0.001 \)). The effects of extracellular pH on \( N_{P_o} \) (Fig. 3) were also pronounced, with \( N_{P_o} = 26 ± 7 \) (n = 13) at pH 7.0 and \( N_{P_o} = 71 ± 12 \) at pH 7.8 (n = 12, \( P < 0.01 \)).

Channel rundown. Patch excision always resulted in partial loss of channel activity. In one experimental series, we found that ~70% of active channels were lost within 2 min after excision (mean number of channels per patch = 40.3 ± 9.5 in cell-attached mode and 12.3 ± 4.1 in excised mode, n = 13). Thereafter, channel activity could be maintained for 20–40 min.

Anion permeability sequence. The ion selectivity of the channel was investigated quantitatively in excised patches containing only a few channels. With the low-NaCl solution in the bath and PS in the pipette, the single-channel current-voltage relation reversed at \( E_o = -41.3 ± 2.6 \) mV (n = 6), indicating anion selectivity (data not shown). The mean \( P_{Na}/P_{Cl} \) was 0.08 ± 0.02 (n = 6). We assessed the selectivity among anions from single-channel current-voltage measurements when most of the Cl− in the bathing solution (i.e., 130 mM) had been replaced by the test anion (Fig. 4). The mean relative permeabilities (Table 1) indicate a permeability sequence of \( Cl^- > I^- > Br^- > NO_3^- > F^- \), which is similar to that determined for CIC-K2, except for the rank of \( I^- \): in the case of CIC-K2, the relative permeability for \( I^- \) is lower (i.e., 0.4) (3).

Sensitivity to pH. On inside-out patches we observed that \( P_o \) was highly dependent on pH (Fig. 5). A similar result has been observed for the Cl− channel in the mouse DCT (23). In contrast to its effect on a Cl− channel in the thick ascending limb (7), intracellular ATP had no stimulatory effect on the CNT Cl− channel in inside-out patches. Furthermore, exposure to ATP did not counteract the partial channel rundown that occurred on excision: the average \( N_{P_o} \) in the presence of 1 mM ATP was 72 ± 1% of that in control (n = 3).

Blockade of the channel. The effects of blockers were tested in inside-out patches using N-methyl-D-glucuronic chloride in the bath and pipette. The results, summarized in Fig. 6A, show that NPPB was more potent than DPC: \( 10^{-4} \) M NPPB and
10⁻³ M DPC inhibited the channel to the same extent, reducing \( N_P \) to 15 ± 4% \((n = 3)\) and 17 ± 7% \((n = 5)\), respectively, at positive voltage. Figure 6B illustrates the inhibition caused by 10⁻⁴ M NPPB and 10⁻³ M DPC. The effects of 10⁻⁴ M NPPB \((n = 3)\) and 10⁻³ M DPC \((n = 2)\) were not dependent on voltage (not shown). DIDS had no clear inhibitory effect \((n = 2; \text{data not shown})\).

A similar channel in the CCD. We consistently found a channel with similar conductance \((9.8 ± 0.7 \text{ pS, } n = 8)\) and \( E_r \) \((2.2 ± 2.4 \text{ mV, } n = 8)\) in cell-attached patches formed on the basolateral membrane of the CCD. For the CCD channel, the ion selectivity was determined using a low-NaCl solution in the bath, and we found that \( E_r \) was -40.6 ± 2.6 mV \((n = 3)\) and \( P_{Na}/P_{Cl} \) was 0.08 ± 0.02 \((n = 3)\). The selectivity among anions was very similar to that found for the CNT Cl⁻ channel (Table 1), indicating that we were dealing with the same channel. Furthermore, similar to the CNT channel, the CCD Cl⁻ channel was sensitive to pHₗ: \( N_P \) values at pH 6.8 and pH 8.0 were 25 ± 6% and 186 ± 32%, respectively, of \( N_P \) at pH 7.4 \((n = 3, \text{data not shown})\).

Cl⁻ channels and K⁺ channels are located in different subsets of cells. During these experiments, we became convinced that the Cl⁻ channels were located in intercalated, rather than principal, cells. This was our impression in the CCD, where principal and intercalated cells can reasonably be differentiated under the microscope (6, 44). It also seemed to hold true for the CNT, but differentiating between the two cell types on the sole basis of visual inspection was not always
practicable in the latter case. Thus, to document further the heterogeneous distribution of Cl⁻ channels, we used patch-clamp conditions, which allowed simultaneous recording of Cl⁻ and K⁺ channels (see Methods) to differentiate between the two cell types. Our rationale was derived from J reports showing that the principal cells have high K⁺ conductance and no (or very low) Cl⁻ conductance, whereas Cl⁻ conductance predominates in intercalated cells (see the introduction), and 2) patch-clamp studies reporting basolateral K⁺ channels in the principal cells of the rat CCD (see Ref. 11 for review).

Using this protocol, we analyzed a total number of 103 patches in the CNT; 52 of these patches were not investigated further, because they showed no clear channel activity \(( n = 7)\), cation channels were present \(( n = 32)\), or complete current-voltage relations could not be established \(( n = 14)\). We found one dominant K⁺ channel in the CNT \((24 of 51 patches, 47\%\)) with a conductance of 38.9 ± 2.6 pS \(( n = 24; Fig. 7A)\). This channel, which is inwardly rectifying (not shown; inward-to-outward conductance ratio = 0.17 ± 0.03, \( n = 7\)), resembles previously described K⁺ channels in the mouse DCT and CTAL \((22, 28)\). Another, presumably K⁺-selective channel with higher inward conductance \((83.2 ± 6.7 pS, n = 4)\) was found on a few occasions \((4 of 51 patches, 8%; Fig. 7B)\). This channel probably corresponds to the high-conductance type reported in the basolateral membrane of the rat CCD \((12, 43)\). K⁺ channels were recorded in 28 of the 51 patches \((55\%)\) but showed simultaneous Cl⁻ channel activity in only 2 cases (i.e., 7% of the patches showed K⁺ channel activity, 4% of the total number of patches). Examples of recordings showing exclusive K⁺ channel activity or mixed K⁺ channel-Cl⁻ channel activity are shown in Fig. 6. Cl⁻ channels \((conductance = 10.9 ± 0.2 pS, E_r = −0.3 ± 0.3 mV)\) were observed with no K⁺ channel in 23 patches \((45\%)\). Thus our experimental data indicate that a vast majority of K⁺ and Cl⁻ channels are located in different cells within the CNT.

We also examined the CCD for the presence of Cl⁻ and K⁺ channels. A total of 60 patches were monitored; 31 of these patches could be analyzed for K⁺ or Cl⁻ channel activity. Most inwardly rectifying K⁺ channels had an inward-slope conductance of ~40 pS \((39.3 ± 4.8 pS, n = 13)\) and, thus,
Fig. 5. Effects of intracellular pH (pHi) on Cl⁻ channel activity. A: Channel current traces showing that channel activity decreased at acidic pH and increased at alkaline pH. Traces were recorded at +50 mV in inside-out configuration, with low-NaCl solution on the inner side of the membrane patch and PS in the pipette. B: Effects of pHi on NPₒ. Each bar is mean of 4 measurements. There were 5–28 Cl⁻ channels per excised patch.

Fig. 6. Effects of Cl⁻ channel blockers. A: NPₒ in the presence of 5-nitro-2-(3-phenylpropylamino)benzoate (NPPB) or diphenylamine-2-carboxylate (DPC). Number of observations is indicated in parentheses. There were 3–29 (11.5 ± 2.1) Cl⁻ channels per patch. Bath contained N-methyl-D-glucanate chloride solution, and pipette solution was PS. B and C: Channel current traces extracted from 1 continuous recording from an inside-out patch showing effects of 10⁻⁴ M NPPB at +60 mV and 10⁻³ M DPC at +80 mV.
corresponded to the intermediate type described in the basolateral membrane of the rat CCD (11, 12, 43, 44). Another channel with a lower conductance of ~20 pS was encountered in a minority of patches that also had the 40-pS channel. K⁺ channels alone were detected in 18 of 31 patches (58%) and Cl⁻ channels alone (conductance ~10.0 ± 0.7 pS, $E_r = -0.7 ± 2.3$ mV) in the other 13 patches (42%). We found no patches that displayed simultaneous K⁺ and Cl⁻ channel activity.

**DISCUSSION**

Several Cl⁻ channels have been identified in the native renal tubule of the mouse by means of the patch-clamp technique. Our laboratory has characterized three channels in the CTAL and DCT; two of these channels have a ~10-pS conductance comparable to that of the channel reported here. The first channel (7, 8, 24), recorded in the CTAL and the late part of the DCT (DCT2), has the following anion selectivity sequence: Br⁻ > NO₃⁻ > Cl⁻ > F⁻. It is sensitive to pH, (8) and is stimulated by ATP, PKA (7), and pyrophosphate (24). The anion selectivity and some properties of this channel, namely, its activation by ATP, are in sharp contrast with the properties of the CNT-CCD Cl⁻ channel. The CNT-CCD Cl⁻ channel most closely resembles the second channel, which was previously investigated in the early and late parts of the DCT (DCT1 and DCT2) (23, 27), and exhibits the following permeability sequence: Cl⁻ ~ I⁻ > Br⁻ ~ NO₃⁻ > F⁻. This channel is inhibited at acidic pH but is not stimulated by ATP. Importantly, the activity of this channel increases when extracellular Ca²⁺ or pH increases (23), a property also found for a Cl⁻ channel reconstituted into artificial membrane bilayers (35). It therefore looks as if the same basolateral Cl⁻ channel is present in DCT1, DCT2, CNT, and CCD.

With use of an antibody that does not discriminate between the two CIC-K channels, it has been shown that CIC-K1 and CIC-K2 are embedded in the basolateral membrane along diverse segments of the renal tubule (24, 37, 38), including the CNT and the CCD. The channel detected in the native CNT and CCD is likely to be a CIC-K channel for the following reasons: 1) It is sensitive to extracellular pH and Ca²⁺ in the same way as CIC-K (3, 41). 2) It has a permeability sequence that is in part similar to that of CIC-K. Indeed, whereas most Cl⁻ channels display an anionic permeability sequence of $\Gamma^- > NO_3^- > Br^- > Cl^- > F^-$, CIC channels, at least those expressed at the plasma membrane, such as CIC-0, CIC-1, and CIC-2, rather, display a sequence of $Cl^- > Br^- > NO_3^- > Cl^-$ for CIC-K1 and $Cl^- > Br^- > NO_3^- = Cl^-$ for CIC-K2). However, this second argument is weakened by the fact that, in our experiments, $\Gamma^-$ is almost as permeant as Cl⁻. We have no explanation for this discrepancy, which can possibly be due to experimental conditions (extracellular substitution for cloned CIC-K channels in the oocyte system vs. intracellular substitution in our experiments).
CIC-K1 and CIC-K2 have been located at the cellular level in the CNT and the CCD (3, 18, 40). On the one hand, using CIC-K1-knockout mice, Kobayashi et al. (18) showed that CIC-K2 is present in type A intercalated cells within the CCD and in principal cells in the CNT. Principal cells in the CNT were identified as those expressing the Na$^+/Ca^{2+}$ exchanger. Kobayashi et al. do not refer to intercalated cells in the CNT. On the other hand, Estevez et al. (3) reported CIC-K and the regulatory subunit of CIC-K, barttin, in intercalated cells within the CCD that possess the basolateral anion exchanger AE1 (type A intercalated cells) and those that do not (type B and non-A non-B). These results indicate that the CIC-K-barttin complex is mainly expressed in intercalated cells but can also be present in principal cells of the CNT, which is consistent with the location of the 10-pS Cl$^-$ channel (see below).

We found Cl$^-$ and K$^+$ channels in distinct patches. Although, strictly speaking, it can be argued that Cl$^-$ and K$^+$ channels are segregated within the same cell membrane, we think that these results indicate distribution in separate cell types, because we were able to observe the two channels in the same patches in the CTAL (see METHODS). In addition, data in the literature indicate that the basolateral K$^+$ channels are located in the principal cells, and we have recorded similar channels: several patch-clamp studies, in which the cell type was determined morphologically, previously demonstrated the presence of three different K$^+$ channels in the principal cells of the CCD, with high ($\sim$80 pS), intermediate (50 pS), and low ($\sim$20 pS) conductance (see Ref. 11 for review). In addition, a heteromeric association of the inwardly rectifying K$^+$ channels Kir 4.1 and Kir 5.1 certainly underlies the intermediate-conductance K$^+$ channel (22), which was predominant in this study. We know that the Kir 4.1 protein is located on the basolateral side in the principal, but not intercalated, cells (15).

Finally, the electrical properties of the CNT and CCD have been investigated using the isolated, microperfused tubule technique. It has been shown that the basolateral membranes of type A and type B intercalated cells (26, 38) predominantly conduct Cl$^-$. Conversely, the basolateral membranes of the principal cells mainly (rabbit CCD) or exclusively (rat CCD) conduct K$^+$ (26, 36, 37). Taken together, these data strongly suggest that the K$^+$ channels in our study were recorded from principal cells and, therefore, that Cl$^-$ channels are mainly located on intercalated cells. However, a minority of cells in the CNT (9% of patches with K$^+$ channels) show simultaneous activity of both channel types. This study does not specifically resolve whether all types of intercalated cells are endowed with Cl$^-$ channels. However, given the high incidence of the Cl$^-$ channel reported here, it is very likely that it is present in the two predominant types: non-A non-B intercalated cells (57% of the intercalated cells in the CNT and 22% in the CCD) and type A intercalated cells (51% of intercalated cells in the CCD and 34% in the CNT) (16). Type B is less frequent and corresponds to $\approx$8 and 16% of intercalated cells in the CNT and CCD, respectively (16).

The functions of the Cl$^-$ channels in intercalated cells have not been investigated directly. Hypotheses that rely on the idea that Cl$^-$ channels optimize HCO$_3^-$ flux mediated by Cl$^-$/HCO$_3^-$ exchange have been advanced (Fig. 7) (10, 38). This would imply that the Cl$^-$ channels help the AE1 Cl$^-$/HCO$_3^-$ exchanger in type A intercalated cells extrude HCO$_3^-$ toward the interstitium by recycling Cl$^-$ across the basolateral membrane, thus ultimately favoring H$^+$ extrusion into the tubule lumen. In type B and non-A non-B intercalated cells, where the Cl$^-$/HCO$_3^-$ exchanger pendrin is located at the luminal membrane (17, 31, 42), net Cl$^-$ absorption may occur via the basolateral Cl$^-$ channel (Fig. 7). The non-A, non-B type is predominant in the CNT (57% of intercalated cells), whereas type A is more common in the CCD (51% of intercalated cells) (16). Another possible function concerns control of the membrane voltage. On the basis of present knowledge, the membranes of the intercalated cells are thought to have low conductance to K$^+$, and it has been suggested that the membrane voltage is mainly controlled by the H$^+$ pump (4). Because intracellular Cl$^-$ concentration can be determined by several ion transporter systems (pendrin, AE1, or the K$^+$-Cl$^-$ cotransporter, depending on the cell type), Cl$^-$ channels in intercalated cells control the membrane potential.

In summary, this study reports a Cl$^-$ channel in the basolateral membranes of CNT intercalated cells that is similar to the previously described DCT1 Cl$^-$ channel (22) in terms of conductance, anion selectivity, pharmacological profile, inhibition by intracellular and extracellular protons, and increased activity at high extracellular Ca$^{2+}$ concentrations. It is probably widely distributed throughout the nephron, because a Cl$^-$ channel with the same conductance and anion selectivity is present in DCT2 (27) and CCD intercalated cells. The present information suggests that the basis of this channel is CIC-K2, but a definite conclusion will be reached only when the properties of recombinant CIC-K2 are known in more detail.

**ACKNOWLEDGMENTS**

The English text was checked by Monika Ghosh. We thank Martine Imbert-Teboul for constant support.

**GRANTS**

A. Nissant and S. Lachheb hold a Ph.D. fellowship from the Ministère de la Recherche, and M. Paulais is an Institut National de la Santé et de la Recherche Médicale researcher.

**REFERENCES**


CHLORIDE CHANNEL IN THE DISTAL NEPHRON


