IGF-1 and insulin exert opposite actions on ClC-K2 activity in the cortical collecting ducts

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Zaika O, Mamenko M, Boukelmoune N, Pochynyuk O. IGF-1 and insulin exert opposite actions on ClC-K2 activity in the cortical collecting ducts. Am J Physiol Renal Physiol 308: F39–F48, 2015. First published October 22, 2014; doi:10.1152/ajprenal.00545.2014.—Despite similar stimulatory actions on the epithelial sodium channel (ENaC)-mediated sodium reabsorption in the distal tubule, insulin promotes kaliuresis, whereas insulin-like growth factor-1 (IGF-1) causes a reduction in urinary potassium levels. The factors contributing to this phenomenon remain elusive. Electrogenic distal nephron ENaC-mediated Na+ transport establishes driving force for Cl− reabsorption and K+ secretion. Using patch-clamp electrophysiology, we document that a Cl− channel is highly abundant on the basolateral plasma membrane of intercalated cells in freshly isolated mouse cortical collecting duct (CCD) cells. The channel has characteristics attributable to the ClC-K2: slow gating kinetics, conductance ~10 pS, voltage independence, Cl−/NO3− anion selectivity, and inhibition/activation by low/high pH, respectively. IGF-1 (100 and 500 nM) acutely stimulates ClC-K2 activity in a reversible manner. Inhibition of P3-kinase (PI3-K) with LY294002 (20 μM) abrogates activation of ClC-K2 by IGF-1. Interestingly, insulin (100 nM) reversibly decreases ClC-K2 activity in CCD cells. This inhibitory action is independent of PI3-K and is mediated by stimulation of a mitogen-activated protein kinase-dependent cascade. We propose that IGF-1, by stimulating ClC-K2 channels, promotes net Na+ and Cl− reabsorption, thus reducing driving force for potassium secretion by the CCD. In contrast, inhibition of ClC-K2 by insulin favors coupling of Na+ reabsorption with K+ secretion at the apical membrane contributing to kaliuresis.

distal nephron; Cl− reabsorption; epithelial transport; urinary K+ excretion

IT IS GENERALLY RECOGNIZED that transport in the distal part of renal tubule, including the connecting tubule and the collecting duct, plays an important role in maintenance of whole body fluid homeostasis by shaping excretion rates of water and electrolytes with urine (32, 40). This part contains two morphologically and functionally distinct cell populations, where principal cells (PC) mediate sodium and water reabsorption and secretion of potassium, and intercalated cells (IC) control acid-base balance and also participate in chloride reabsorption (40, 44). Activity of the epithelial sodium channels (ENaC) located to the apical membrane of PC underlies electrogenic Na+ transport in PC, which, in turn, provides driving force for luminal K+ exit via the renal outer medulla potassium channels (40). Cl− reabsorption occurs via both paracelluar (through tight junctions) and transcellular (across IC) routes (44).

The initial step of the transcellular Cl− reabsorption is largely mediated by the electroneutral Cl−/HCO3− transporter Slc26a4 (pendrin) in base secreting B type of IC and Slc4a11 transporter acting as an electrogenic Cl−/HCO3− exchanger or a Cl− channel in acid secreting A type of IC on the apical side (46). Recent experimental evidence indicates that basolateral Cl− export to the interstitium in the cortical collecting duct (CCD) is mediated by the activity of the chloride ClC-K2 channel present in both A and B types of IC (18, 28, 42). ClC-K2 (ClC-Kb in humans) is predominantly expressed in the kidney and shares substantial homology with other members of ClC channels (13, 42, 43). In addition to the CCD, ClC-K2 is also present in other parts of the distal renal tubule, specifically in the thick ascending limb (TAL) and the distal convoluted tubule (DCT) (18). Interestingly, gain-of-function polymorphism ClC-KbT481S in humans is associated with elevated blood pressure due to augmented renal salt retention (12). This suggests that the channel possibly plays an essential role in mediating Cl− reabsorption in the distal parts of renal tubule. Consistently, ClC-K2 loss-of-function mutations lead to salt-wasting phenotype of Bartter’s syndrome type III associated with hypotension (39). However, little is known about mechanisms controlling function of the channel in different segments of the renal tubule, and specifically in the CCD, expressing ClC-K2.

Insulin and structurally related insulin growth factor-1 (IGF-1) have been long recognized to play a role in controlling renal function (2, 8). Both hormones trigger activation of P3-kinase (PI3-K)- and mitogen-activated protein kinase (MAPK)-dependent pathways via predominant binding to respective insulin and IGF-1 receptors expressed at both apical and basolateral sides along the renal tubule (2, 7, 8). In the CCD, insulin and IGF-1 stimulate ENaC-mediated sodium reabsorption through a PI3-K-dependent mechanism (41). Acute intravenous IGF-1 injection results in a significant reduction in the fractional Na+ excretion in humans (6). In patients with acromegaly, augmented circulating IGF-1 levels result in antinatriuresis and hypertension, which can be corrected with ENaC inhibitor amiloride (14, 15). In contrast, the effects of insulin are very dependent on plasma glucose and K+ levels and often do not lead to salt retention and elevation in blood pressure (5, 20). Furthermore, IGF-1 reduces renal potassium excretion (6), whereas insulin can promote kaliuresis (5), particularly when plasma K+ levels are exogenously clamped (9, 36). The molecular details of the distinct effects of insulin and IGF-1 on urinary excretion patterns are not clear. Despite the fact that transcellular reabsorption of Na+ and Cl− is carried out in different cell types in the CCD, accumulating experimental evidence suggests that PC and IC often cooperate to promote reabsorption of NaCl with little change in net pH balance (reviewed in Ref. 44). Thus, genetic ablation of pendrin causes respective decreases in ENaC activity and protein abundance, contributing to a lower blood pressure and
decreased renal salt retention (17, 33). On the other side, pendrin overexpression in IC leads to activation of sodium reabsorption without significant changes in trans-epithelial voltage and potassium excretion (11). In contrast, augmentation of Na⁺ transport in the PC only will result in stimulation of both potassium secretion and, to a lesser extent, paracellular Cl⁻ reabsorption (44). It is not clear whether CIC-K2-mediated basolateral chloride conductance contributes to the separation of Cl⁻ and K⁺ fluxes in the CCD.

In this study, we tested the effects of insulin and IGF-1 on the basolateral Cl⁻ conductance in mouse CCD. Using patch-clamp electrophysiology, we report that highly abundant 10-pS anion-selective channel at the basolateral plasma membrane demonstrates properties attributable to CIC-K2. Nanomolar concentrations of IGF-1 reversibly stimulated channel activity in a PI3-K-dependent manner. In contrast, insulin inhibited CIC-K2 activity in CCD cells via a mechanism dependent on MAPK activation. We propose that the opposite effects of insulin and IGF-1 on Cl⁻ transport in the CCD may, at least partially, underlie distinct patterns of urinary electrolyte excretion in response to the hormones.

MATERIALS AND METHODS

Reagents and animals. All chemicals and materials were from Sigma (St. Louis, MO), VWR (Radnor, PA), and Tocris (Ellisville, MO) unless noted otherwise and were at least of reagent grade. Animal use and welfare adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals following protocols reviewed and approved by the Animal Care and Use Committees of the University of Texas Health Science Center at Houston. For experiments, male C57BL/6J mice (Charles River Laboratories, Wilmington, MA) 6–10 wk old were used. Animals were maintained on standard rodent regimen (Purina, #5001) and had free access to tap water.

Tissue isolation. The procedure for isolation of the CCDs suitable for electrophysiology is a modification from the protocols described previously (23, 24, 26, 47). Mice were killed by CO₂ administration for electrophysiology is a modification from the protocols described respectively. The current-voltage (I–V) relationships were obtained by calculating the permeability ratio for Cl⁻ and NO₃⁻ as described by Goldman, Hodgkin, and Katz.

Ion selectivity. The permeability ratio for Cl⁻ and NO₃⁻ was calculated using the equation of Goldman, Hodgkin, and Katz. For this, the reversal potential (Eᵦᵥ) was determined in inside-out patch-clamp experiments, where 150 mM KCl in the recording pipette was substituted with 150 mM KNO₃.

Basolateral membrane voltage measurements. To monitor real-time changes in membrane voltage, CCD cells were studied under current-clamp mode using the perforated-patch technique. Freshly made Amphotericin-B, 400 μM (Enzo Life Sciences, Farmingdale, NY) was dissolved in the pipette solution containing 150 mM KCl, 2 mM MgCl₂, 10 mM HEPES (pH 7.35) by ultrasonication. Electrical recordings were made once the access resistance from the pipette to the cell interior fell to <15 MΩ, usually 5–10 min after achieving a pipette-to-membrane seal resistance of 5–10 GΩ.

Data analysis. All summarized data are reported as means ± SE. In paired experiments, data from before and after treatment were compared using the paired t-test. Data from unpaired experiments were compared with a Student’s (2-tailed) t-test or a one-way ANOVA as appropriate. P < 0.05 was considered significant.

RESULTS

Determination of molecular identity of the basolateral Cl⁻ channel in CCD cells. Previous studies suggest the presence of anion conductance on the basolateral membrane of IC (28, 30). We first employed patch-clamp electrophysiology in a cell-attached configuration in freshly isolated enzymatically treated mouse CCD to perform functional characterization of this conductance. Using patch pipette containing 150 mM KCl, we observed a highly abundant Cl⁻ channel in ~40% of patches. Typical patch-clamp recordings at different pipette potentials and the I–V relationship of the channel with slow gating kinetics in CCD cells are shown in Fig. 1A and 1B, respectively. The Eᵦᵥ was ~0 mV and the estimated channel conductance was 11.5 ± 0.3 pS. Equimolar substitution of K⁺ to Na⁺ and Li⁺ in the pipette solution, had no measurable effect on single channel conductance and Eᵦᵥ (data not shown). In contrast, replacement of Cl⁻ with acetate induced rightward shift of the I–V curve by ~20 mV (Fig. 1B, gray trace). This indicates an anion-selective nature of the channel. The majority of patches have more than five channels that make evaluation of the Pₒ not always accurate. Thus, we assessed Pₒ of the channel only in patches containing fewer than five channels. Figure 1C demonstrates no apparent voltage de-
dependence of channel $P_o$ at the tested pipette potentials from $-100$ to $+40$ mV.

We next quantitatively determined anion selectivity of the recorded channel upon excision of the patch and controlling solute composition at both sides. Figure 2A demonstrates respective $I-V$ relationship in cell-attached (black trace) and inside-out (gray trace) configurations when the majority of $Cl^{-}$ in the pipette was replaced with $NO_3^-$. The calculated permeability ratio $P_{Cl}/P_{NO_3} = 1.46$ (see MATERIALS AND METHODS). Furthermore, the leftward shift of $\sim 15$ mV in the $I-V$ upon excitation suggests that the channel predominates in the IC, which are known to have much more depolarized basolateral membrane potential than PC (27).

We next tested whether basolateral $Cl^{-}$ channels participate in setting resting membrane potential in IC. For this, we directly monitored changes in the basolateral membrane volt-

Fig. 1. Functional properties of ClC-K2-like channels abundantly expressed on the basolateral membrane of cortical collecting duct (CCD) cells. A: representative current traces of single channel activity recorded from the same patch at different pipette potentials, as indicated. A c denotes closed nonconductive state. B: average current-voltage ($I-V$) relationship of the unitary current amplitude for channels similar to that shown in A when patch pipette contains 150 mM KCl (black trace) and 150 mM KAcetate (gray trace). Number of experiments is also indicated. C: dependence of open probability ($P_o$) of the channel from voltage applied to the recording pipette. For each condition, $P_o$ was estimated over a time span of at least 180 s.

Fig. 2. Anion selectivity and contribution to the basolateral voltage of ClC-K2-like channel. A: average $I-V$ relationship of the unitary current amplitude for the basolateral $Cl^{-}$ channels when patch pipette contains 150 mM KNO3 in cell-attached (black trace) and excised inside-out (gray trace) configurations. Number of experiments is also indicated. B: representative continuous voltage trace monitoring basolateral membrane potential in an intercalated cell (IC) in the control, upon application of a broad spectrum $Cl^{-}$ channel inhibitor 100 μM NPPB and 150 mM NaCitrate. Application times are shown with respective bars on top.
age using current-clamp configuration upon application of broad spectrum Cl⁻ channel inhibitors NPPB (100 μM) or DIDS (100 μM). Previous reports showed that similar concentrations of the antagonists drastically reduced ClC-K2 activity in renal tubule cells (1, 28). We focused on IC having values of basolateral membrane voltage of ~−10 mV, in contrast to PC having basolateral membrane voltage of ~−70 mV (47). NPPB treatment has no measurable effect on the basolateral membrane voltage (n = 5), whereas replacement of bath NaCl for NaCitrate elicits prominent depolarization, as expected (Fig. 2B). Application of DIDS also has no effect on membrane potential (n = 4, data not shown). These results suggest that either ClC-K2 does not directly participate in controlling basolateral membrane potential or ClC-K2 inhibition can be compensated by other membrane transporter proteins.

We next probed regulation of Cl⁻ channel activity by pH. An acute increase in extracellular pH from 7.35 to 8.0 induces pronounced augmentation of channel activity as demonstrated on the representative current trace in Fig. 3A. Washout with control saline (pH 7.35) returns activity of the canal to control values. Furthermore, application of acidic extracellular media (pH 5.0) drastically reduces channel $P_o$ in a reversible manner (Fig. 3B). Figure 3C summarizes the effect of extracellular pH on the activity of Cl⁻ channels in paired experiments similar to that demonstrated in Fig. 3, A and B. The mean values of $P_o$ are $0.05 \pm 0.01$, $0.30 \pm 0.04$, and $0.64 \pm 0.02$ for pH 5.0, 7.35, and 8.0, respectively. We concluded that the channel is highly pH sensitive. Alkalic pH potentiates Cl⁻ channel, whereas acidic pH decreases channel activity.

All defined properties: slow channel kinetics (Fig. 1A), single channel conductance (Fig. 1B), independence from applied voltage (Fig. 1C), expression in IC, anion permeability pattern (Fig. 2A), and pH sensitivity (Fig. 3) of the basolateral Cl⁻ channel in CCD cells are consistent with those reported for the ClC-K2 channel, which was previously shown to be functionally expressed in distal tubular segments, including the CCD (22, 28, 45).

IGF-1 stimulates ClC-K2 channel activity in a PI3-K-dependent manner. Previous reports document that IGF-1 receptors are expressed at the basolateral membrane of CCD (2, 35). ClC-K2, in tandem with HCO₃⁻/Cl⁻ exchangers Slc26a4 (pendrin) and Slc4a11, is thought to be involved in
the net Cl⁻ reabsorption in IC of the CCD (44). IGF-1 stimulates Na⁺ reabsorption by increasing ENaC activity in PC (41). Thus, we next determined whether IGF-1 also targets ClC-K2 to promote Cl⁻ transport in the CCD. Basolateral application of 100 nM IGF-1 acutely stimulates ClC-K2 activity in a reversible manner, as demonstrated by the representative continuous current trace in Fig. 4A. The summary graph of changes in ClC-K2 $P_o$ in paired experiments upon treatment with IGF-1 is shown in Fig. 4B. The mean values of $P_o$ are 0.31 ± 0.04, 0.55 ± 0.06, and 0.41 ± 0.06 in the control, during IGF-1 (100 nM) application, and washout, respectively ($n = 7$). Application of a higher IGF-1 concentration (500 nM) yields virtually identical results (Fig. 4C). The mean values of $P_o$ are 0.35 ± 0.05, 0.59 ± 0.06, and 0.42 ± 0.07 in the control, during IGF-1 (500 nM) application, and washout, respectively ($n = 5$).

Stimulation of IGF-1 receptors generally leads to activation of a PI3-K-dependent cascade (8). Therefore, we next tested whether inhibition of PI3-K affects regulation of ClC-K2 activity by IGF-1 in CCD cells. Application of a PI3-K inhibitor, LY294002 (20 μM, 3 min), has no measurable effect on ClC-K2, as summarized in Fig. 5. However, this treatment abolishes stimulation of ClC-K2 activity by IGF-1. The mean values of $P_o$ are 0.31 ± 0.06, 0.33 ± 0.07, 0.31 ± 0.07, and 0.30 ± 0.06 in the control, after LY294002 (20 μM), during application of IGF-1 (100 nM) in the continued presence of the PI3-kinase antagonist, and washout, respectively ($n = 5$). Overall, we concluded that IGF-1 stimulates ClC-K2 in a PI3-K-dependent manner.

Insulin inhibits ClC-K2 via stimulation of a MAPK-dependent cascade. We next aimed to investigate regulation of ClC-K2 by insulin, which also gives a stimulatory effect on ENaC-mediated sodium reabsorption in the CCD (10, 31). In contrast to IGF-1, application of insulin (100 nM) greatly decreased ClC-K2 activity and the effect was reversed upon washout of the hormone, as demonstrated in the representative continuous current trace in Fig. 6A. The summary graph of changes in ClC-K2 $P_o$ in paired experiments upon treatment with insulin is shown in Fig. 6B. The mean values of $P_o$ are 0.34 ± 0.04, 0.11 ± 0.02, and 0.24 ± 0.04 in the control, during insulin (100 nM) application, and washout, respectively ($n = 7$).
DISCUSSION

In this study, we provide the first functional evidence that basolateral Cl\(^-\) conductance in CCD cells is under direct hormonal control by IGF-1 and insulin. We further put forward the hypothesis that the opposing effects of the aforementioned hormones on the basolateral Cl\(^-\)K2 chloride channel participate in separation of Cl\(^-\) and K\(^+\) fluxes stimulated by ENaC-mediated Na\(^+\) reabsorption depending on physiological needs. This, in turn, suggests a possible mechanism that contributes to different patterns of urinary excretion of electrolytes caused by IGF-1 and insulin (Fig. 8). Specifically, we propose that activation of Cl\(^-\)K2 channels by IGF-1 and subsequent stimulation of a PI3-K-dependent pathway facilitates net NaCl retention (Fig. 8A), whereas inhibition of the channel by insulin via a MAPK-dependent mechanism creates favorable conditions for coupling of Na\(^+\) and K\(^+\) transport in the CCD (Fig. 8B), therefore, elevating excretion of K\(^+\) with urine.

Using patch-clamp technique, we routinely monitor the activity of the anion channel at the basolateral membrane of CCD cells, which demonstrates all the molecular signature characteristics of Cl\(^-\)K2 (28). This includes ~10-pS conductance, slow gating, independence of \(P_o\) from applied voltage, Cl\(^-\) > NO\(_3\^-\) selectivity, and pH sensitivity. Our observations resonate with previous work by Teulon's group (28) that recorded a channel with virtually identical characteristics in the CNT/CCD region of the mouse renal tubule. It also appears that the channel is expressed in cells having greatly depolarized basolateral membrane potential (Fig. 2A), which are IC. Indeed, previous studies in perfused tubules suggest low-K\(^+\) conductance in the basolateral membrane of IC (as opposed to PC having predominantly K\(^+\) exiting mechanisms) and major Cl\(^-\) conductance (19, 27, 38). An anion channel with similar properties was reported to be functionally expressed in the DCT and the TAL (22, 28, 30, 45). The expression pattern of the channel is consistent with the immunofluorescent detection of Cl\(^-\)K2 in mouse kidney sections (18). Overall, the existing evidence strongly supports the view that Cl\(^-\)K2 activity mediates basolateral Cl\(^-\) conductance in IC of CCD. It should be noted, although, that the final conclusion can be made only after ablation of Cl\(^-\)K2 in this segment using genetic tools.

One of the most striking features of Cl\(^-\)K2 is its functional expression levels. Thus far, this is the most abundant channel recorded with patch clamping in the CCD cells. The majority of cell-attached patches have more than five simultaneously active Cl\(^-\)K2 channels, which makes it difficult to reliably estimate channel \(P_o\) in all cases. While not exactly precise, patch-clamp studies indicate that functional expression levels of Cl\(^-\)K2 in CCD region are no less or even possibly higher than those in the DCT and TAL (22, 30, 45). It is proposed that Cl\(^-\)K2 participates in Cl\(^-\) reabsorption in the distal renal tubule, starting from TAL (18). Inhibition of Cl\(^-\)K2 in vivo promotes diuresis (21) and loss-of-function mutations in Cl\(^-\)K2 result in salt-wasting phenotype of Barter’s syndrome type III (39). However, the levels of Cl\(^-\)K2 in CCD are far more than necessary to perform equimolar reabsorption of NaCl at this site considering functional expression levels of ENaC (23, 25, 34), the major route for electrogenic Na\(^+\) transport, and similar conductance of both channels. This argues that Cl\(^-\)K2 is likely contributing to processes other than Cl\(^-\) reabsorption, such as regulatory volume increase/decrease and possibly establishing basolateral membrane voltage. While our data (Fig. 2B) fail to demonstrate the direct effect of Cl\(^-\)K2 activity on membrane voltage, this possibility cannot be ruled out because compensatory mechanisms likely exist. The absence of currently available selective Cl\(^-\)K2 inhibitors does not also exclude the scenario that nonspecific adverse effects of NPPB and DIDS may contribute to the observed negative results. In addition, remarkable pH sensitivity of Cl\(^-\)K2 indicates that the channel may participate in regulation of acid-base balance in IC. Further studies are necessary to carefully examine these possibilities.

Our observation that insulin and IGF-1 exert opposing effects on the basolateral Cl\(^-\)K2 channels in the CCD cells is somehow surprising from the first glance. Insulin and IGF-1 have substantial structural similarity and IGF-1 receptor is also very similar to insulin receptor (16, 37). Both receptors possess tyrosine kinase activity and involve activation of PI3-K and MAPK pathways (8). Despite this, insulin and IGF have clearly distinct physiological functions. Activation of IGF-1 receptors is primarily important for growth and development, whereas
insulin receptors are viewed as metabolic mediators (16). Furthermore, insulin and IGF-1 have much lower affinity (up to 500-fold) for the respective counterpart receptor (7). We found that activation of distinct intracellular signaling pathways, namely PI3-K-dependent (Fig. 5) and MAPK-dependent (Fig. 7), mediates regulation of ClC-K2 activity by IGF-1 and insulin, respectively. This indicates that different receptors are involved in conferring opposite effects on ClC-K2 activity in response to the hormones. A recent study demonstrates that similar concentrations of IGF-1 (up to 300 nM) cause inhibition of basolateral Cl−/H+ channel (presumably ClC-K2) in TAL cells via activation of PI3K-AKT-mTOR pathway (45). It was proposed that IGF-1-induced inhibition of trans-epithelial Cl− absorption in the TAL would decrease oxygen consumption in the medullary TAL, thus contributing to a beneficiary role of IGF-1 during ischemia-reperfusion injury (29). However, intrarenal infusions of similar IGF-1 concentrations induce antidiuresis and antinatriuresis (4, 6), which is consistent with augmented net NaCl reabsorption by renal tubule. Indeed, stimulatory effects of IGF-1 on sodium-transporting proteins, NKCC2 in the TAL and ENaC in CCD, are reported (3, 41). We did observe that 100 and 500 nM IGF-1 elicit virtually identical reversible increases in the activity of basolateral ClC-K2 channel in CCD cells (Fig. 4, B and C), which likely facilitates salt retention at this site. It is possible that different sets of membrane receptors for IGF-1 and insulin are present in TAL and CCD, to convey segment-specific actions of the hormones. In support of this view, insulin in concentrations for up to 500 nM had no effect on ClC-K2 activity in the TAL (45). It should be noted that micromolar insulin concentration did inhibit ClC-K2, pointing that the effect is mediated by IGF-1R.

According to the classical view, the distal nephron, and specifically CCD, is the site where potassium secretion is coupled with ENaC-mediated sodium reabsorption (40). ENaC activity creates a favorable driving force for K+ to exit from principal cells which, in turn, is a major contributor for urinary potassium levels. Mineralocorticoid aldosterone is the principal ENaC activator (40). However, the substantial portion of patients with elevated circulating aldosterone levels does not develop renal potassium wasting and hypokalemia (48). This suggests that Na+ and K+ fluxes in the CCD can occur independently based on physiological needs. Modulation of trans-cellular Cl− reabsorption might be one of the possible
Fig. 7. Insulin decreases ClC-K2 activity via a MAPK-dependent pathway. A: summary graph of changes in ClC-K2 P_o in paired cell-attached patch-clamp experiments in the control, upon treatment with PI3-K inhibitor LY294002 (20 μM), insulin (100 nM) in the continued presence of the inhibitor, and following washout. *Significant decrease vs. control. B: summary graph of changes in ClC-K2 P_o in paired cell-attached patch-clamp experiments in the control, upon treatment with MEK inhibitor U0126 (5 μM), insulin (100 nM) in the continued presence of the inhibitor, and following washout.

Fig. 8. Principal scheme of regulation of ClC-K2 activity in CCD cells by IGF-1 (A) and insulin (B). Solid bold and dashed thin arrows represent dominant and minor ion transporting pathways, respectively.
duced by these hormones. IGF-1, by stimulating ENaC in PC and CIC-K2 channels in IC, elicits cooperative NaCl reabsorption and, therefore, a reduction of potassium secretion by the CCD. In contrast, insulin by stimulating ENaC but inhibiting CIC-K2 speeds up ion fluxes in PC cells only (i.e., coupling of Na+ reabsorption with K+ secretion).

In summary, this manuscript provides the first observation of direct effects of insulin and IGF-1 on the basolateral Cl−/H+ conductance, and specifically on the basolateral CIC-K2 channel, in the IC of CCD via distinct intracellular signaling pathways. It is possible that modulation of trans-cellular Cl− reabsorption by controlling CIC-K2 activity may provide a physiologically relevant mechanism enabling dissociation of sodium reabsorption from potassium secretion at this site.

**REFERENCES**


