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Guanylin and uroguanylin regulate electrolyte transport in isolated human cortical collecting ducts

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Guanylin and uroguanylin regulate electrolyte transport in isolated human cortical collecting ducts.

Background. Guanylin and uroguanylin link intestinal and renal electrolyte and water transport. Their function in intestine is well studied, but renal actions are less understood. Uroguanylin concentrations are increased in patients with chronic renal failure, nephrotic syndrome, or those on dialysis. Guanylate cyclase C (GC-C) is the receptor first described for these peptides. In guanylate cyclase C–deficient mice guanylin- and uroguanylin-induced renal natriuresis, kaliuresis, and diuresis are retained.

Methods. Effects of guanylin and uroguanylin on principal cells of human cortical collecting ducts (CCD) isolated from kidneys after tumor nephrectomy were investigated. Reverse transcription-polymerase chain reaction (RT-PCR), slow whole-cell patch-clamp, and microfluorimetric analysis of intracellular Ca^{2+} were used. Here we present first functional measurements of isolated human CCD.

Results. Principal cells of CCD were identified by the amiloride-induced hyperpolarization of principal cells (-3.8 ± 0.3 mV) ($N = 52$). Cells depolarized upon guanylin or uroguanylin (each 10 nmol/L) by 3.3 ± 0.8 mV ($N = 12$) and 3.4 ± 0.5 mV ($N = 18$), respectively, but were hyperpolarized by 8Br-cyclic guanosine monophosphate (cGMP) (100 µmol/L) (-3.0 ± 0.2 mV) ($N = 4$). mRNA for GC-C was not detected in CCD. Effects of both peptides were inhibited by Ba^{2+} (1 mmol/L) or phospholipase A_2 (PLA_2) inhibition (AACOCF$_3$) (5 µmol/L).

Conclusion. These findings suggest a new cGMP- and GC-C–independent but PLA_2-dependent signaling pathway for these peptides in the kidney. Most likely guanylin and uroguanylin inhibit luminal K^+ channels of principal cells of human CCD via this pathway. This depolarization of principal cells consequently reduces the driving force of Na^+ and water reabsorption, explaining natriuresis and diuresis caused by these peptides.

Key words: human, principal cells, electrophysiology, K conductances, natriuretic peptides.

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The existence of intestinal natriuretic peptides was proposed by the work of Lennane et al in 1975 [1] who showed that salt taken orally induces higher natriuresis than an intravenous salt load. Increased salt ingestion leads to secretion of guanylin and/or uroguanylin into the intestinal lumen [2, 3] and blood stream [4]. Guanylin and uroguanylin induce natriuresis, kaliuresis, and diuresis in the kidney [4, 5]. Natriuresis produced by oral salt load is decreased in uroguanylin-deficient mice [6], which suggests that uroguanylin and guanylin are intestinal natriuretic peptides. Both activate guanylate cyclase C (GC-C) which was first described as receptor for the heat-stable enterotoxin of *Escherichia coli* (STa) [7]. Mimicking the physiologic effects of guanylin and uroguanylin, STa which has a higher affinity to GC-C produces secretory diarrhea [8]. Activation of GC-C increases intracellular cyclic guanosine monophosphate (cGMP) and activates protein kinase G (PKG), which stimulates the cystic fibrosis transmembrane regulator (CFTR) leading to secretion of Cl^-, HCO_3^-, and water in the intestine [9–11].

GC-C–deficient mice (GC-C−/−) show resistance to STa in the intestine, but guanylin, uroguanylin, and STa still cause natriuresis, kaliuresis, and diuresis suggesting the existence of a GC-C–independent signaling pathway in the kidney [12]. In a human proximal tubule cell line, we recently demonstrated that guanylin, uroguanylin, and STa activate two signaling pathways: one involving GC-C leading to increased cellular cGMP, the other involving the activation of a pertussis toxin–sensitive G protein [13]. Both pathways lead to regulation of K^+ conductances in these human proximal tubule cells.

The involvement of guanylin and uroguanylin in kidney diseases is evident from the fact that uroguanylin concentrations in plasma and urine are increased in patients with chronic renal failure [14] or nephrotic syndrome [15]. Guanylin and uroguanylin concentrations are also increased in patients undergoing dialysis [14]. This could be a consequence of kidney damage and therefore reduced renal metabolism and excretion of these peptides

and/or guanylin and uroguanylin are directly involved in the development of renal diseases.

Guanylin and uroguanylin also play an important role in the development of intestinal tumors. Shailubahai et al [16] showed that oral application of uroguanylin leads to a decrease in number and size of polyps in mice which develop intestinal polyposis, proposing uroguanylin as a novel drug in the therapy of intestinal tumors. Further, guanylin and uroguanylin play a significant physiologic and pathophysiologic role also in the reproductive and lymphatic systems [17] and the lungs [18]. Uroguanylin, intravenous or inhaled, inhibited bronchoconstriction and mucus secretion in airways of guinea pig [19]. These results led to the conclusion that uroguanylin is a possible drug in asthma therapy.

Guanylin and uroguanylin were proposed as novel drugs for different human diseases. Thus, further understanding of detailed renal actions of these peptides especially in the human kidney is urgently needed. In this study we show, for the first time, cellular actions of guanylin and uroguanylin in isolated tubule segments. Furthermore, these data are the first functional data obtained from isolated human nephron segments. We demonstrate that guanylin and uroguanylin change a K^+ conductance of principal cells of cortical collecting ducts (CCD) and thereby alters the driving force for Na^+ and water reabsorption. The pathway for guanylin and uroguanylin in principal cells of human CCD is GC-C–independent and involves an activation of phospholipase A_2 (PLA_2).

METHODS

Human tissues

Healthy cortical pieces from human kidneys were obtained from patients undergoing tumor nephrectomies. In this study, 47 human kidneys during more than 3 years were used. Thirty-eight kidneys were from male patients with an average age of 62 ± 2 years (ranging from 32 to 80 years). Nine kidneys were from female patients with an average age of 70 ± 5 years (ranging from 49 to 95 years). The ethics committee of the Universitätsklinikum Münster approved the procedure, and written consent was obtained from each patient. Renal tissue was separated and stored in modified Ringer's solution (described below) on ice in the operation theater as soon as possible. After cooled transfer to the laboratory the isolation procedure was started immediately.

Isolation of human nephron segments

Large quantities of individual tubules were isolated for reverse-transcription-polymerase chain reaction (RT-PCR) analysis as described before [20]. Small human cortical kidney pieces were incubated in minimal essential medium (MEM) plus 5 mmol/L glycin, 480 IU/mL trypsin inhibitor from soybean, 50 IU/mL DNAse, and 533 IU/mL collagenase type 2 (Worthington Biochemical Corp., Lakewood, NJ, USA) for 60 to 100 minutes at 37°C with aeration (95% O_2 and 5% CO_2), resulting in a tubule suspension. Specific nephron segments were collected in MEM with 5 mmol/L glycin under 50× magnification at 4°C. For patch clamp experiments and measurements of intracellular Ca^{2+}, CCD were isolated using the procedure described for rat kidney [21] and modified for human kidneys as follows. Cortical pieces from human kidneys were incubated at 37°C with MEM plus 5 mmol/L glycin, 26 mmol/L $NaHCO_3$, 429 IU/mL collagenase type 4 (Worthington Biochemical Corp.) and 4 IU/mL protease for 45 minutes. CCD segments were collected in the above medium without enzymes but in addition containing 50 g/L albumin and 1 μmol/L deoxycorticosterone acetate (DOCA) under 50× magnification at 4°C (DOCA solution). To enable sealing of patch clamp pipettes with the plasma membrane and at the same time allow access of the bath solution to the luminal membrane CCD clusters were prepared using a Ca^{2+}-free solution (MEM) [5 mmol/L glycin and 5 mmol/L ethyleneglycol tetraacetate (EGTA)] for 4 minutes at 4°C [22]. Further, for patch clamp experiments, clusters were incubated at 37°C in DOCA solution for at least 2 hours before the experiments were started to increase the Na^+ conductance of principal cells of CCD.

RT-PCR

Human kidney pieces, isolated nephron segments (approximately 40 mm), glomeruli (400 pieces) were lysed in a 4 mol/L guanidium chloride buffer and total RNA was isolated using the RNeasy kit (Qiagen, Hilden, Germany). cDNA was synthesized by reverse transcriptase (Promega, Heidelberg, Germany) and subjected to PCR reactions. For detecting mRNA for GC-C, we used the primers 5′-GTATTGCCCTCTTTCTCTTTTCC-′ and 5′-TTCCAGCCCCTCATTCAC-3′ (product length 538 bp, annealing temperature 53°C) and for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (used as positive control for the PCR reaction) 5′-CTGCCCCCTCTGCTGATG-3′ and 5′-GTCCACCACCCTGTTGCTGT-3′ (614 bp, 56°C). Reaction conditions were as follows: 2 minutes at 94°C, 30 seconds at 53°C or 56°C, and 1 minute at 72°C, 1 cycle; 30 seconds at 94°C, 30 seconds at 53°C or 56°C, and 1 minute at 72°C, 30 cycles; and 30 seconds at 94°C, 30 seconds at 53°C or 56°C, and 10 minutes at 72°C, 1 cycle. PCR reaction products were analyzed by agarose gel electrophoresis and verified by sequence analyses (Sequence Laboratories, Göttingen, Germany).

Patch clamp studies

CCD clusters were fixed with a glass holding pipette in the experimental chamber mounted on an inverted microscope (Axiovert 10) (Zeiss, Göttingen, Germany). A modified Ringer's solution containing 145 mmol/L NaCl, 1.6 mmol/L K_2HPO_4, 0.4 mmol/L KH_2PO_4, 5 mmol/L D-glucose, 1 mmol/L $MgCl_2$, and 1.3 mmol/L calcium gluconate (pH 7.4) was used as bath solution. Experiments were performed at 37°C with a bath perfusion rate of 10 mL/min at a bath volume of 500 μL. Patch clamp pipettes were filled with a solution containing 95 mmol/L potassium gluconate, 30 mmol/L KCl, 4.8 mmol/L Na_2HPO_4, 1.2 mmol/L NaH_2PO_4, 5 mmol/L D-glucose, 1.3 mmol/L calcium gluconate, 1.03 mmol/L $MgCl_2$, and 1 mmol/L adenosine triphosphate (ATP) (pH 7.2). To this solution 160 μmol/L nystatin was added to permeabilize the membrane under the pipette. Membrane potential (V_m) was measured with a patch clamp amplifier [23]. Due to the high electrical coupling between principal cells and the fact that seals were only obtained with pipette resistances > 10 MΩ reproducible recordings of effects on whole cell currents were not possible.

Fluorescence measurements of intracellular Ca^{2+}

The same experimental setup as described above but in addition equipped with a fluorescence detecting system with a photon-counting tube (Hamamatsu H 3460-04, Herrsching, Germany) was used for measurements of intracellular Ca^{2+}. CCD clusters were incubated with fura2-acetoxymethyl ester (1 μmol/L) for 30 minutes and excited at 340 nm, 380 nm, and 360 nm. The fluorescence ratio (340/380) was calculated. Experiments were controlled and results analyzed with a computer system as described before [24].

Materials

MEM was obtained from Gibco (Karlsruhe, Germany). Guanylin and uroguanylin were purchased from Peptide Institute (Osaka, Japan). $AACOCF_3$ was purchased from Biomol (Hamburg, Germany). All other chemicals were obtained from Merk (Darmstadt, Germany) or Sigma (Taufkirchen, Germany).

Statistics

Student paired *t* test was used with each effect compared with its own averaged pre- and postcontrol values. Data are given as mean values ± SEM with the number of experiments given in brackets. $P < 0.05$ was set as significance level.

RESULTS

Electrophysiologic properties of human CCD

V_m of principal cells of human CCD was measured by the slow-whole-cell patch clamp technique after incubation in DOCA solution for at least 2 hours. Principal cells were identified by hyperpolarizations caused by the inhibitor of epithelial Na^+ channels amiloride (1 μmol/L) (−4.1 ± 0.4 mV) ($N = 53$) (Fig. 1A), as the epithelial Na^+ channel (ENaC) is present in CCD only in principal cells [23]. Basal V_m of human principal cells of CCD was –66 ± 2 mV) ($N = 64$). Increasing the extracellular K^+ concentration by 15 mmol/L depolarized cells by 24 ± 1 mV) ($N = 64$) (Fig. 1A), which corresponds to a relative K^+ conductance of approximately 70%. The general K^+ channel blocker Ba^{2+} (1 mmol/L) depolarized principal cells by 20 ± 2 mV) ($N = 18$). The frequency distribution of basal V_m is shown in Figure 1B and the correlation of basal V_m with the depolarizations caused by this increase in the K^+ concentration in Figure 1C. The classic second messenger involved in regulation of CCD water and Na^+ conductance in the rat is cAMP [23, 25]. The membrane permeable analogue 8Br-cAMP (100 μmol/L) depolarized principal cells of human CCD (2.3 ± 0.7 mV) ($N = 6$).

Effects of guanylin and uroguanylin

Guanylin and uroguanylin (10 nmol/L) led to depolarizations of V_m of principal cells of human CCD (guanylin 3.3 ± 0.8 mV) ($N = 12$) (uroguanylin 3.4 ± 0.5 mV) ($N = 18$). These effects were immediate, reversible and reached their maximum within 1 to 2 minutes (Fig. 2A and B). These effects of guanylin and uroguanylin on principal cells are summarized in Figure 2c.

GC-C is not present in human principal cells

cGMP is the second messenger for guanylin and uroguanylin in the intestine [26, 27]. Therefore, we tested if cGMP is involved in the signaling pathway of guanylin and uroguanylin in principal cells of human CCD. Opposite to the depolarizations caused by guanylin and uroguanylin, 8Br-cGMP (100 μmol/L), a membrane permeable analogue of cGMP, hyperpolarized principal cells (−3.0 ± 0.2 mV) ($N = 4$) (Fig. 2B and C). As shown before [13] mRNA for GC-C was detected in human proximal tubules but was absent in human CCD (Fig. 3). These results exclude cGMP and GC-C as part of the signaling pathway for guanylin and uroguanylin in human principal cells.

Guanylin and uroguanylin do not alter cellular Ca^{2+} ($[Ca^{2+}]_i$)

An additional possible signaling partway for guanylin and uroguanylin is Ca^{2+} and protein kinase C (PKC) [28]. To determine the involvement of Ca^{2+} signaling in the effects of guanylin and uroguanylin in human CCD we measured $[Ca^{2+}]_i$ with the fluorescent dye fura2- ester. Guanylin or uroguanylin did not significantly increase $[Ca^{2+}]_i$ in human CCD (Fig. 4A), whereas prostanglandin E_2 (PGE_2) increased $[Ca^{2+}]_i$.

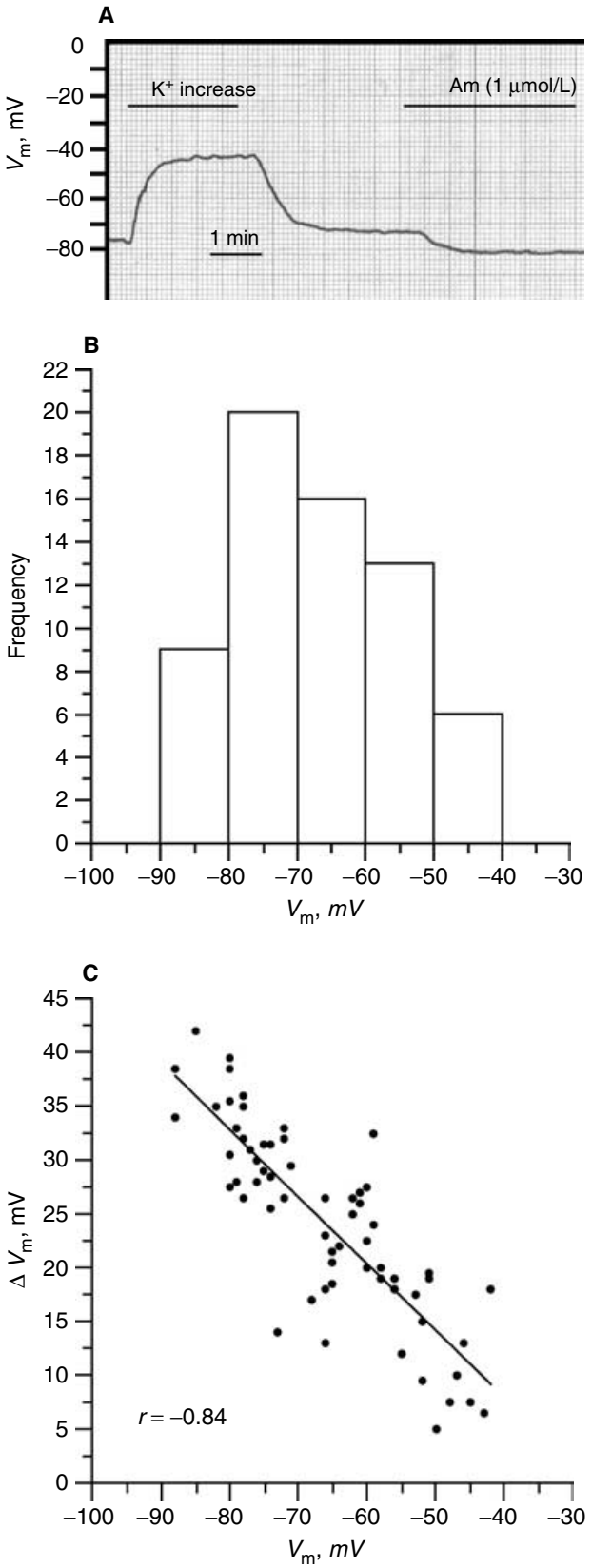

Fig. 1. General electrophysiologic characteristics of principal cells of human cortical collecting ducts (CCD). V_m was measured by the slow-whole-cell patch clamp technique after incubation in deoxycorticosterone acetate (DOCA) solution for at least 2 hours. (*A*) Original trace showing depolarizations caused by an increase in the K^+ concentration from 3.6 to 18.6 mmol/L and the identification of a principal cell by the amiloride (Am) (1 µmol/L) induced hyperpolarization. (*B*) Frequency distribution of basal V_m which shows a Gaussian-like distribution. (*C*) Correlation between V_m and depolarizations caused by an increase in extracellular K^+ from 3.6 mmol/L to 18.6 mmol/L ($r = -0.84$).

Again as shown before for the rat [24] such changes in $[Ca^{2+}]_i$ do not significantly change V_m of principal cells (ΔV_m by PGE_2 (-1.0 ± 0.3 mV) ($N = 3$). A summary of these results is given in Figure 4B. Thus, changes of V_m caused by guanylin and uroguanylin did not involve changes in $[Ca^{2+}]_i$ in human principal cells and vice versa.

Role of K^+ channels in the action of guanylin and uroguanylin

Changes of V_m could be caused by changes in Na^+, K^+, or Cl^- conductances. To identify the involvement of K^+ conductances in the action of guanylin and uroguanylin the general K^+ channel blocker Ba^{2+} was used. Depolarizations caused by guanylin or uroguanylin were completely blocked by Ba^{2+} indicating the involvement of K^+ conductances in the signaling pathway of these peptides (Fig. 5).

Inhibition of the PLA_2 blocked depolarizations caused by uroguanylin

Arachidonic acid is covalently linked to glycerol in the middle position of phospholipides and is liberated by enzymatic PLA_2-mediated hydrolysis. Arachidonic acid inhibits renal outer medullary K^+ channel (ROMK) channels located at the luminal membrane of principal cells [29]. Here arachidonic acid (10 µmol/L) led to depolarizations of V_m (10.0 ± 0.8 mV) ($N = 3$). Since the effects of guanylin and uroguanylin are due to changes in K^+ conductances the involvement of PLA_2 in the signaling pathway of these peptides was tested using $AACOCF_3$, an inhibitor of PLA_2. Depolarizations caused by uroguanylin (10 nmol/L) were inhibited by 5 µmol/L $AACOCF_3$ (Fig. 6). These results suggest an involvement of PLA_2 in the signaling pathway of guanylin and uroguanylin in human principal cells.

DISCUSSION

The current understanding of the main physiologic function of guanylin and uroguanylin is apparently to couple renal electrolyte transport to intestinal transport and increase renal Na^+ excretion after ingestion of high amounts of salt [6]. Their relevance for the K^+ homeostasis of the body and its renal regulation has not been discussed yet. The signaling pathways, receptors, and localization of actions of these peptides in the kidney are still poorly understood. As these peptides seem to be

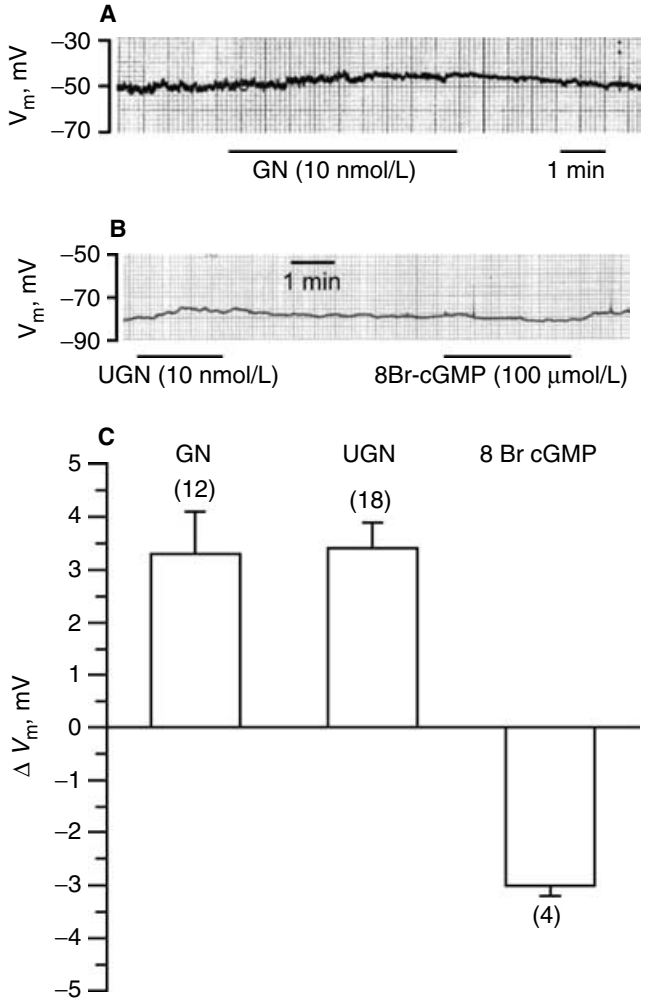

Fig. 2. Effects of guanylin (GN) and uroguanylin (UGN) on V_m of principal cells of human cortical collecting ducts (CCD). V_m was measured by the slow whole-cell patch clamp technique after incubation in deoxycorticosterone (DOCA) solution for at least 2 hours. (*A*) Original trace showing the depolarization of a principal cell caused by guanylin (10 nmol/L). (*B*) Original trace showing the depolarization caused by uroguanylin (10 nmol/L) and the opposite effect of 8Br-cyclic guanosine monophosphate (cGMP) (100 µmol/L). (*C*) Summarized effects of guanylin, uroguanylin, and 8Br-cGMP on human principal cells (mean ± SEM, number of experiments given in brackets).

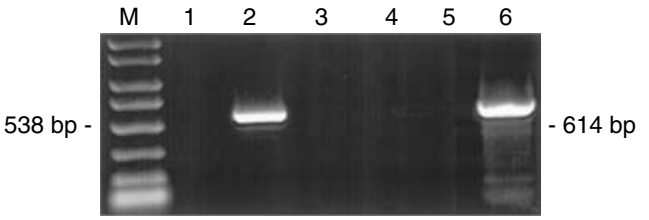

Fig. 3. Reverse transcription-polymerase chain reaction (RT-PCR) analysis of the expression of mRNA for guanylate cyclase C (GC-C), which is not present in human cortical collecting ducts (CCD), but present in proximal tubules. M, marker; lane 1, glomeruli; lane 2, proximal tubules; lane 3, thick ascending limbs; lane 4, cortical collecting ducts; lane 5, negative control (no cDNA); and lane 6, positive control [glyceraldehyde-3-phosphate dehydrogenase (GAPDH)]. Representative sample of three similar experiments.

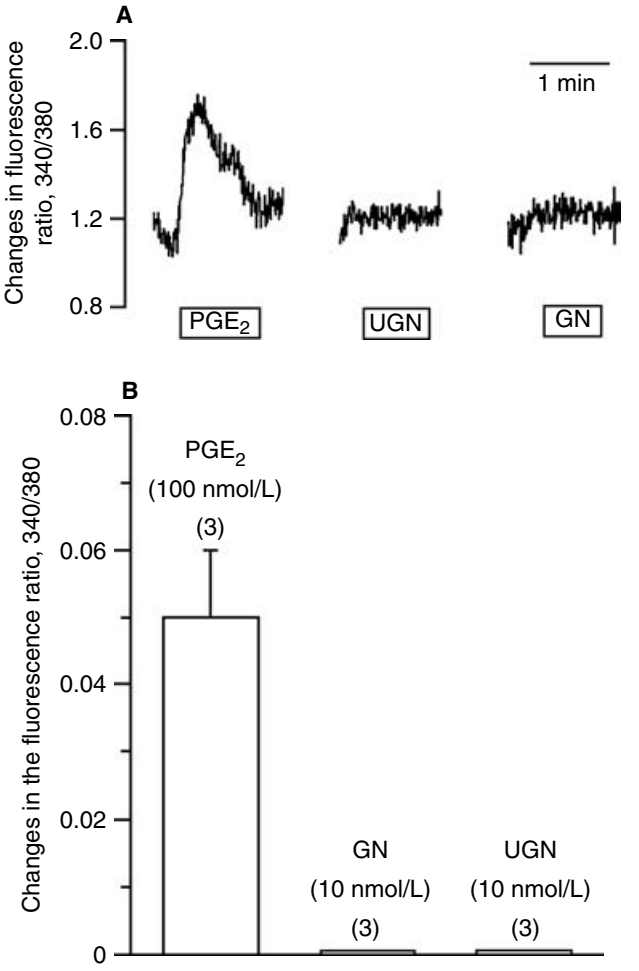

Fig. 4. Effects of guanylin (GN) and uroguanylin (UGN) on $[Ca^{2+}]_i$ in human cortical collecting ducts (CCD). CCD were incubated with fura2-acetoxymethyl ester (1 µmol/L) for 30 min. (*A*) Original trace showing the absence of effects of guanylin (10 nmol/L), uroguanylin (10 nmol/L), and the effect of prostaglandin E_2 (PGE_2) (100 nmol/L) as positive control. (*B*) Summary of the effects (mean ± SEM, number of experiments given in brackets).

involved in various renal and extrarenal diseases [14–16, 19], they have been discussed for possible therapeutic use [16, 19]. Therefore, detailed understanding of the mechanisms of their renal actions is urgently needed. Besides earlier reports on saliuresis and diuresis caused by these peptides in vivo or in vitro in the rat [5, 12], we recently described, for the first time, renal effects of guanylin and guanylin as well as of STa on the cellular level in a human proximal tubule cell line [13]. The results from this cell line suggest that these peptides reduce reabsorption along the proximal tubule. Clearance studies suggest the collecting duct as another possible site of guanylin and uroguanylin actions because this segment plays the

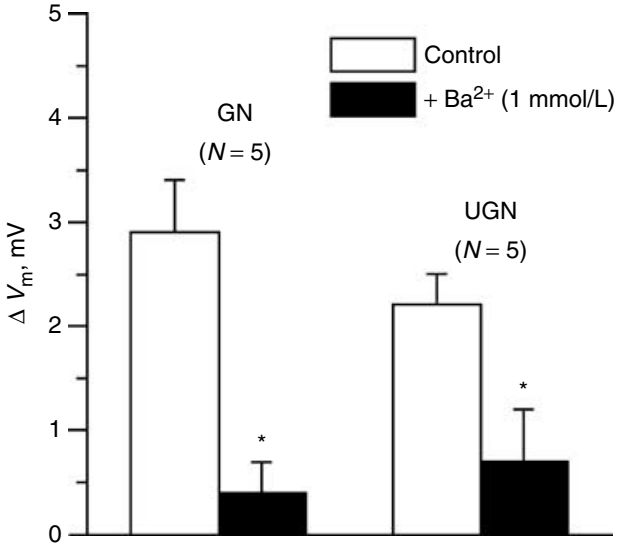

Fig. 5. Role of K^+ conductances in the action of guanylin (GN) and uroguanylin (UGN). V_m was measured by the slow whole-cell patch clamp technique after incubation in deoxycorticosterone acetate (DOCA) solution for at least 2 hours. Depolarizations caused by both peptides (10 nmol/L each) were blocked by Ba^{2+} (1 mmol/L). $^*P <$ 0.05 between effects of guanylin or uroguanylin in the presence of Ba^{2+} compared to control effects (mean ± SEM, paired experiments, number of experiments given in brackets).

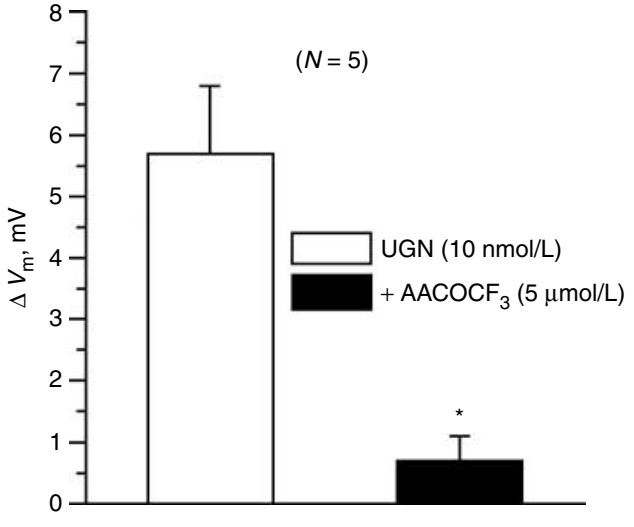

Fig. 6. Effects of $AACOCF_3$, an inhibitor of phospholipase A_2 (PLA_2) on uroguanylin (UGN) effects. V_m was measured by the slow whole-cell patch clamp technique after incubation in deoxycorticosterone (DOCA) solution for at least 2 hours. $^*P < 0.05$ between effects of uroguanylin in the presence of $AACOCF_3$ compared to control effects (mean ± SEM, paired experiments, number of experiments given in brackets).

major role in the final regulation of Na^+, K^+, and water excretion.

In this study we present, for the first time, functional data of principal cells of freshly isolated human CCD using patch clamp and fura2 analysis of V_m and $[Ca^{2+}]_i$. The accessibility of fresh human renal tissue for such functional measurements is obviously very limited and using healthy tissue surrounding renal tumors offers a rather unique opportunity to isolate individual nephron segments also for such studies. The isolation of CCD from those tissue samples was modified from the method already described for the rat [21]. Basal V_m of human principal cells was not different compared to basal V_m of rat principal cells measured with the same technique [21]. V_m clearly correlates with the relative contribution of the K^+ conductance as predicted from the general cellular model of principal cells which possesses K^+ conductances in the basolateral and luminal membrane and a luminal Na^+ conductance [23]. Whereas in the human proximal tubule cell line (IHKE-1) guanylin and uroguanylin activated two signaling pathways leading to depolarizations or hyperpolarizations of V_m [13], these peptides only depolarized principal cells of human CCD (Fig. 2). Therefore, we assumed the existence of one receptor/signaling pathways for guanylin and uroguanylin in the CCD. Since cGMP is the second messenger for these peptides in the intestine [26, 27] and at least for one pathway in the proximal tubule [13] we determined if cGMP plays a role in the signaling pathway of guanylin or uroguanylin in principal cells of human CCD. Similar to the rat [21], cGMP hyperpolarized human principal cells of CCD (Fig. 2B and C). Even more, mRNA for GC-C, the first receptor described for these peptides in the intestine [26, 27] and also expressed in human proximal tubules [13], was neither present in human (Fig. 3) nor in mouse nor in rat CCD [30, 31]. These results suggest the existence of a cGMP- and GC-C–independent signaling pathways for guanylin and uroguanylin in the human CCD. This conclusion also explains why in GC-C–deficient mice guanylin still induces natruiuresis and diuresis. The latter further demonstrates that apparently the collecting duct is more relevant for the renal actions of these peptides compared to the proximal tubule.

Since Ca^{2+} and PKC were suggested as a possible additional and cGMP-independent signaling pathway for these peptides in the intestine [28] we, therefore, tested if Ca^{2+} as a second messenger is involved in the effect of those peptides in human principal cells. However, guanylin and uroguanylin did not change $[Ca^{2+}]_i$. This is in line with the absence of effects of other Ca^{2+}-activating agonists on ion conductances in the rat CCD reported before [24] and shown here as well for the human CCD.

Depolarizations of principal cells can be the result of an increase of the Na^+ conductance or a decrease of K^+ conductances. While the first would result in deceased natriuresis, the latter by decreasing the driving force for Na^+ and water reabsorption would lead to natriuresis and diuresis, as observed for guanylin and uroguanylin [4, 5,

12]. To identify the involvement of K^+ conductances in the action of guanylin and uroguanylin the general K^+ channel blocker Ba^{2+} was used. Depolarizations caused by both peptides were inhibited in the presence of Ba^{2+} (Fig. 3) suggesting indeed an involvement of changes in K^+ conductances in the signaling pathways of guanylin and uroguanylin in human CCD principal cells as shown before by us for the human proximal tubule cell line [13]. The kind of preparation and analysis used here does not allow to directly attribute these effects to either basolateral or luminal K^+ channels. The secretory K^+ channel of the CCD is ROMK and ROMK-deficient mice show natriuresis, kaliuresis, and diuresis [32] like animals stimulated with guanylin or uroguanylin. This proposes ROMK channels as possible final targets for guanylin and uroguanylin. The second K^+ channel expressed in the luminal membrane, the maxi-K^+ channel, is not active under basal conditions and therefore can be excluded [33]. Furthermore, it is known that arachidonic acid inhibits these ROMK channels located at luminal membrane of principal cells of the rat [29] and therefore, it was not surprising that in our study arachidonic acid depolarized human principal cells. Stimulation of PLA_2 sets free arachidonic acid from phospholipides, structural components of each plasma membrane. Inhibition of PLA_2 with AA-$COCF_3$ blocked depolarizations caused by uroguanylin suggesting the involvement of PLA_2, arachidonic acid and consequently ROMK channels in the signaling pathway of these peptides in human principal cells of CCD. In mouse CCD, receptors for guanylin and uroguanylin are localized in the luminal membrane (own unpublished observations). Uroguanylin is found in the urine and mRNA for guanylin is expressed in CCD [31]. Taken together these data suggest luminal action of guanylin and uroguanylin also in human CCD. A reduction in the K^+ conductance of principal cells by inhibition of ROMK channels and the consequent depolarizations will reduce the driving force for Na^+ and consecutive water reapsorption and thus, could at least in part explain the natriuresis and diuresis seen in vivo with these peptides. Such a hormonal regulation of ROMK channels could be important in kidney diseases when K^+ secretion is impaired.

Guanylin and uroguanylin mediate their renal action via a signaling pathway which is GC-C– and cGMP-independent and thus, different from their signaling pathway in the intestine. The described signaling pathway, which involves PLA_2 and arachidonic acid in human CCD needs to be further investigated to evaluate its specific role in pathophysiology such as nephritic syndrome. These findings, however, further suggest so far unknown mechanisms for an involvement of guanylin or uroguanylin in disorders of other organs like lungs, intestine or reproductive organs. It is for sure necessary to be considered when guanylin or uroguanylin are proposed for the therapy of intestinal tumors [16] or asthma [19].

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REFERENCES

1. Lennane RJ, Peart WS, Carey R, Shaw J: A comparison of natriuresis after oral and intravenous sodium loading in sodium-depleted rabbits: Evidence for a gastrointestinal or portal monitor of sodium intake. *Clin Sci Mol Med* 49:433–436, 1975
2. Carrithers SL, Jackson BA, Cai WY, *et al*: Site-specific effects of dietary salt intake on guanylin and uroguanylin mRNA expression in rat intestine. *Regul Pept* 107:87–95, 2002
3. Kita T, Kitamura K, Sakata J, Eto T: Marked increase of guanylin secretion in response to salt loading in the rat small intestine. *Am J Physiol* 277:G960–G966, 2000
4. Greenberg RN, Hill MJ, Crytzer J, *et al*: Comparison of effects of uroguanlyin, guanylin and *Escherichia coli* heat-stable enterotoxin STa in mouse intestine and kidney: Evidence that uroguanylin is an intestinal natriuretic hormone. *J Investig Med* 45:276–283, 1997
5. Fonteles MC, Greenberg RN, Monteiro HSA, *et al*: Natriuretic and kaliuretic activites of guanylin and uroguanylin in the isolated perfused rat kidney. *Am J Physiol* 275:F191–F197, 1998
6. Lorenz JN, Nieman M, Sabo J, *et al*: Urouganylin knockout mice have increased blood pressure and impared natriuretic response to enteral NaCl load. *J Clin Invest* 122:1244–1254, 2003
7. Schulz S, Green KC, Yuen PST, Garbers DL: Guanylyl cyclase is a heat-stable enterotoxin receptor. *Cell* 63:941–948, 1990
8. Field M, Graf LH, Laird WJ, Smith PL: Heat-stable enterotoxin of *Escherichia coli*: In vitro effects on guanylate cyclase activity, cyclic GMP concentration, and ion transport in small intestine. *Proc Natl Acad Sci USA* 75:2800–2804, 1978
9. Cuthbert AW, Hickman ME, MacVinish LJ, *et al*: Chloride secretion in response to guanylin in colonic epithelia from normal and transgenic cystic fibrosis mice. *Br J Pharmacol* 112:31–36, 1994
10. Guba M, Kuhn M, Forssmann W-G, *et al*: Guanylin strongly stimulates rat duodenal HCO_3^- secretion proposed mechanism and comparison with other secretagogues. *Gastroenterology* 111:1558–1568, 1996
11. Joo NS, London RM, Kim HD, *et al*: Regulation of intestinal Cl^- and HCO_3^- secretion by uroguanylin. *Am J Physiol* 274:G633–G644, 1998
12. Carrithers SL, Ott CE, Hill MJ, *et al*: Guanylin and uroguaylin induce natriuresis in mice lacking guanylyl cyclase-C receptor. *Kidney Int* 65:40–53, 2004
13. Sindic A, Basoglu C, Cerci A, *et al*: Guanylin, uroguanylin, and heat-stable euterotoxin activate guanylate cyclase C and/or a pertussis toxin-sensitive G protein in human proximal tubule cells. *J Biol Chem* 277:17758–17764, 2002
14. Kinoshita H, Fujimoto S, Nakazato M, *et al*: Urine and plasma levels of uroguanylin and its molecular form in renal diseases. *Kidney Int* 52:1028–1034, 1997
15. Kinoshita H, Fujimoto S, Fukae H, *et al*: Plasma and urine levels of uroguanylin a new peptide in nephrotic syndrome. *Nephron* 81:160–164, 1999
16. Shailubhai K, Yu HH, Karunanandaa K, *et al*: Uroguanylin treatment suppresses polyp formation in the *Apc* $^{Min/+}$ Mouse and induces apoptosis in human colon adenocarcinoma cells via cyclic GMP. *Cancer Res* 60:5151–5157, 2000
17. Jaleel M, London RM, Eber SL, *et al*: Expression of the receptor guanylyl cyclase C and its lignds in reproductive tissues of the rat: A potential role for a novel signaling pathway in the epididymis. *Biol Reprod* 67:1975–1980, 2002
18. Range SP, Holland ED, Basten GP, Knox AJ: Regulation of

guanosine 3′:5′-cyclic monophosphate in ovine tracheal epithelial cells. *Br J Pharmacol* 120:1249–1254, 1997
19. Ohbayashi H, Yamaki K: Both inhalant and intravenous uroguanylin inhibit leukotriene C4-induced airway changes. *Peptides* 21:1467–1472, 2000
20. Schafer J.A., Watkins ML, Li L, *et al*: A simplified method for isolation of large numbers of defined nephron segments. *Am J Physiol* 273:F650–F657, 1997
21. Hirsch J, Schlatter E: K^+ channels in the basolateral membrane of rat cortical collecting duct are regulated by a cGMP-dependent protein kinase. *Pflügers Arch* 429:338–344, 1995
22. Schlatter E, Fröbe U, Greger R: Ion conductances of isolated cortical collecting duct cells. *Pflügers Arch* 421:381–387, 1992
23. Schlatter E, Schafer JA: Electrophysiological studies in principal cells of rat cortical collecting tubules. ADH increases the apical membrane Na^+-conductance. *Pflügers Arch* 409:81–92, 1987
24. Ankorina-Stark I, Haxelmans S, Schlatter E: Receptors for bradykinin and prostaglandin E_2 coupled to Ca^{2+} signalling in rat cortical collecting duct. *Cell Calcium* 22:269–275, 1997
25. Reif MC, Troutman SL, Schafer JA: Sodium transport by rat cortical collecting tubule. Effects of vasopressin and deoxycorticosterone. *J Clin Invest* 77:1291–1298. 1986
26. Currie MG, Fok KF, Kato J, *et al*: Guanylin: An endogenous activator of intestinal guanylate cyclase. *Proc Natl Acad Sci USA* 89:947–951, 1992
27. Hamra FK, Forte LR, Eber SL, *et al*: Uroguanylin: structure and activity of a second endogenous peptide that stimulates intestinal guanylate cyclase. *Proc Natl Acad Sci USA* 90:10464–10468, 1993
28. Ganguly U, Chaudhury AG, Basu A, Sen PC: STa-induced translocation of protein kinase C from cytosol to membrane in rat entrocytes. *FEMS Microbio Lett 2* 04:65–69, 2001
29. Macica CM, Yang Y, Hebert SC, Wang WH: Arachidonic acid inhibits activity of cloned renal K^+ channel, ROMK1. *Am J Physiol* 271:F588–F594, 1996
30. Hirsch JR, Kruhøffer M, Herter P, *et al*: Cellular localization, membrane distribution and possible function of guanylyl cyclase A and 1 in collecting duct of rat. *Cardiovas Res* 51:553–561, 2001
31. Potthast R, Ehler E, Scheving LA, *et al*: High salt intake increases uroguanylin expression in mouse kidney. *Endocrinology* 142:3087–3097, 2001
32. Lu M, Wang T, Yan Q, *et al*: Absence of small conductance K^+ channel (SK) activity in apical membranes of thick ascending limb and cortical collecting duct in ROMK (Batter's) knockout mice. *J Biol Chem* 277:37881–37887, 2002
33. Schlatter E, Haxelmans S, Hirsch J, Leipziger J. pH dependence of K^+ conductances of rat cortical collecting duct principal cells. *Pflügers Arch* 428:631–640, 1994