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Calcium and Magnesium: Low Passive Permeability and Tubular Secretion in the Mouse Medullary Thick Ascending Limb of Henle's Loop (MTAL)

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Abstract Recent studies from our laboratory have shown that in the mouse and rat nephron Ca^{2+} and Mg^{2+} are not reabsorbed in the medullary part of the thick ascending limb (mTAL) of Henle's loop. The aim of the present study was to investigate whether the absence of transepithelial Ca^{2+} and Mg^{2+} transport in the mouse mTAL is due to its relative low permeability to divalent cations. For this purpose, transepithelial ion net fluxes were measured by electron probe analysis in isolated perfused mouse mTAL segments, when the transepithelial potential difference (PD_{te}.) was varied by chemical voltage clamp, during active NaCl transport inhibition by luminal furosemide. The results show that transepithelial Ca^{2+} and Mg^{2+} net fluxes in the mTAL are not driven by the transepithelial PD_{te}. At zero voltage, a small but significant net secretion of Ca^{2+} into the tubular lumen was observed. With a high lumen-positive PD_{te} generated by creating a transepithelial *bath-to-lumen* NaCl concentration gradient, no Ca^{2+} and Mg^{2+} reabsorption was noted; instead significant and sustained Ca^{2+} and Mg^{2+} net secretion occurred. When a lumen-positive PD_{te} was generated in the absence of apical furosemide, but in the presence of a transepithelial *bath-to-lumen* NaCl concentration gradient, a huge Ca^{2+} net secretion and a lesser Mg^{2+} net secretion, not modified by ADH, were observed. Replacement of Na^+ by K^+ in the lumen perfusate induced, in the absence of PD_{te} changes, important but reversible net secretions of Ca^{2+} and Mg^{2+}. In conclusion, our results indicate that the passive permeability of the mouse mTAL to divalent cations is very low and not influenced by ADH. This nephron segment can secrete Ca^{2+} and Mg^{2+} into the luminal fluid under conditions which elicit large lumen-positive transepithelial potential differences. Given the impermeability of this epithelium to Ca^{2+} and Mg^{2+}, the secretory processes would appear to be of cellular origin.

Key words: Medullary thick ascending limb — Ca^{2+} transport — Mg^{2+} transport — Electron microprobe analysis — Passive permeability — ADH

Introduction

Earlier micropuncture studies of the rat nephron have shown that the loop of Henle plays a key role in calcium and magnesium homoestasis [30]. Approximately 25% of the filtered Ca^{2+} load and about 70% of that of Mg^{2+} are reabsorbed in the loop of Henle. Since descending limbs and thin ascending limbs are known to have low Ca^{2+} and Mg^{2+} permeabilities [21, 31], the thick ascending limb of Henle's loop (TAL) must therefore be an important site for Ca^{2+} and Mg^{2+} reabsorption. Recent in vitro microperfusion studies of isolated nephron segments have revealed that in mice [41] and rats [23] only the cortical portion of the thick ascending limb (cTAL) is able to reabsorb Ca^{2+} and Mg^{2+}. Furthermore, it has been shown that several peptide hormones, including ADH and PTH, stimulate Ca^{2+} and Mg^{2+} reabsorption only in this part of the thick ascending limb [9, 40]. The mechanism of Ca^{2+} reabsorption in the cTAL has been studied extensively. Previous studies from our laboratory of the mouse cTAL [6] and studies from other laboratories of the rabbit cTAL [3, 34, 35] have shown that Ca^{2+} is reabsorbed passively in this nephron segment, very probably via the paracellular shunt pathway, the driving force being the lumen-positive voltage. In some of these experiments the spontaneous transepithelial voltage was experimentally varied by generating either transepithelial *bath-to-lumen* or *lumen-to-bath* NaCl concentration gradients in the absence of active NaCl transport. When the transepithelial voltage was lumen-

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positive, net Ca^{2+} transport was in the absorption direction and when it was lumen-negative, the direction of the Ca^{2+} net flux was reversed and Ca^{2+} net secretion was observed. At zero voltage, no Ca^{2+} net reabsorption was measurable and in some instances even a secretory Ca^{2+} net flux was recorded [6, 42]. With respect to Mg^{2+}, only one study [34] in addition to ours [6] demonstrated a passive reabsorption of this ion in the cTAL.

The divalent cation transport properties of the medullary thick ascending limb (mTAL) have not been so extensively studied as those of the cortical segment. Whether this nephron segment transports divalent cations or not is still under discussion. Although Friedman [11] and Suki et al. [37] concluded that Ca^{2+} was passively transported in the mouse and rabbit mTAL, this conclusion was not supported by studies from our [6, 23] and other laboratories [3, 4] which demonstrated without ambiguity that, at least in the mouse and rat, there is no measurable Ca^{2+} and Mg^{2+} net transport in the mTAL segment.

To answer the question of why the mouse mTAL, unlike the cTAL, does not reabsorb divalent cations, we investigated the Ca^{2+} and Mg^{2+} permeability properties of medullary segments in several experimental situations. Our results indicate that Ca^{2+} and Mg^{2+} transport in the mouse mTAL is not correlated with the transepithelial voltage, suggesting that the passive permeability to Ca^{2+} and Mg^{2+} is very low, which thus explains the absence of any measurable transepithelial cation net transport in this nephron segment. Indeed we found that even in the presence of a high lumen-positive voltage, which should have increased passive transepithelial ion reabsorption, Ca^{2+} was secreted into the tubular lumen. The cellular origin of this secretory process must be envisaged.

Materials and Methods

Medullary thick ascending limbs of Henle's loop (mTAL) of 20 to 30-day old white female Swiss mice were dissected and perfused in vitro, as originally described by Burg et al. [5] and modified by Greger [16]. Tubular segments were obtained from the upper part of the cortex from glomeruli of superficial nephrons. The dissected tubules were transferred to a perfusion chamber which was mounted on the stage of an inverted microscope (Zeiss IM 35, Oberkochem, Germany) and perfused by gravity. After the beginning of their perfusion, tubules were allowed to equilibrate for about 15 min before the flux measurements were started. The flux studies were carried out on tubules perfused at slow and constant perfusion rates (about 2 $nl.min^{-1}$), to achieve a significant decrease of the electrolyte concentrations of the luminal perfusate. Tubular fluid was collected as follows: for each experimental period, the fluid was withdrawn into siliconized pipettes over periods of 30 min, each of which was divided into three collection periods of 10 min, the collected samples separated from each other by water-saturated oil columns. The flux values obtained for each element (Na^+, Cl^-, Ca^{2+}, Mg^{2+}) during these 10-min periods were averaged. All experiments were carried out at 37°C. The bath flow rate was 10–20 $ml.min^{-1}$, to ensure a rapid and complete change of the bath solutions.

Transepithelial Ion Net Flux Measurements

The concentrations of Na, Cl, Ca, Mg, P and S ($mmol.l^{-1}$) in the perfused solutions and the collected tubular fluids were measured by electron probe analysis [24]. Briefly, 0.1 nl droplets of tubular fluid, bath solution, perfusate and standard solutions were deposited under water-saturated oil on a beryllium block which was then immersed for 60 sec in chloroform to remove the oil. The droplets were then allowed to dry in the air. Probable contamination of the collected tubular fluid by the bath solution was suspected when the P content of the tubular fluid (present only as phosphate in the luminal perfusate) decreased significantly and when the S content (present only as HEPES, buffering the peritubular solution) of the same solution simultaneously increased. The tubular samples which were contaminated were discarded. The collection rate ($nl.min^{-1}$) was evaluated by measuring the collected volume with a constriction pipette and dividing this volume by the collection time. Since the mouse thick ascending limb is almost impermeable to water [ref.29, and unpublished data from our laboratory], absolute rates of Na^+, Cl^-, Ca^{2+} and Mg^{2+} net transport (J_x, $pmol.min^{-1}.mm^{-1}$) were calculated as: $J_x = V(Cp\text{-}C_c)/L$, where V is the collection rate ($nl.min^{-1}$), C the concentration ($mmol.1^{-1}$) of the element X in the perfused (p) and collected (c) fluid and L the length (mm) of the perfused tubule. Positive net flux values indicate net reabsorption, negative ones net secretion. The transepithelial potential difference (PD_{te}) was measured at the perfusion end of the tubule [14]. When asymmetrical solutions were used in bath and lumen, appropriate corrections for the recorded PD_{te} were made. Liquid junction potentials were measured in separate experiments with free-flowing electrodes.

Chemicals and Solutions

The following solutions were used (concentration in $mmol.l^{-1}$):

Symmetrical Conditions

Lumen: 150 NaCl, 1.6 K_2HPO_4, 0.4 KH_2PO_4, 1.0 $CaCl_2$, 1.0 $MgCl_2$

Bath: 150 NaCl, 3.6 KCl, 5.0 N-[2-hydroxylethyl]piperazine-N′-[2-ethansulfonic acid] (HEPES), 1.0 $CaCl_2$, 1.0 $MgCl_2$, 5.0 D-glucose

Asymmetrical Conditions

Generation of a transepithelial *lumen-to-bath* directed NaCl concentration gradient:

Lumen: 150 NaCl, 1.6 K_2HPO_4, 0.4 KH_2PO_4, 1.0 $CaCl_2$, 1.0 $MgCl_2$

Bath: 50 NaCl, 200 mannitol, 3.6 KCl, 5.0 N-[2-hydroxylethyl]piperazine-N′-[2-ethansulfonic acid] (HEPES), 1.0 $CaCl_2$, 1.0 $MgCl_2$, 5.0 D-glucose

Generation of a hypotonic transepithelial *bath-to-lumen* directed NaCl concentration gradient:

Lumen: 50 NaCl, 1.6 K_2HPO_4, 0.4 KH_2PO_4, 1.0 $CaCl_2$, 1.0 $MgCl_2$

Bath: 150 NaCl, 3.6 KCl, 5.0 N-[2-hydroxylethyl]piperazine-N′-[2-ethansulfonic acid] (HEPES), 1.0 $CaCl_2$, 1.0 $MgCl_2$, 5.0 D-glucose

The pH of all solutions was adjusted to 7.4.

Inhibitors

Furosemide was obtained from Sigma Chemical, St. Louis, MO. The drug was used at a concentration of 10^{-4} $mol.l^{-1}$ in the lumen perfusate and was dissolved in the luminal solution immediately prior to use.

Hormones

Antidiuretic hormone (ADH, arginine vasopressin, Sigma Chemical St Louis, MO) was added to the bath solution at a concentration of 10^{-10} mol.l^{-1}. In our previous studies of cTAL and mTAL segments of the mouse [39, 41] it was shown that this concentration elicited maximal effects on the transepithelial NaCl and Ca^{2+} and Mg^{2+} transport rates.

Experimental Procedures

This study comprised four experimental series of flux measurements carried out on 35 isolated perfused tubules.

In the first series the passive permeability of medullary TAL segments to Ca^{2+} and Mg^{2+} was explored. For this purpose, two kinds of experiment were performed. In the first one, transepithelial net fluxes of Na^+, Cl^-, Ca^{2+}, and Mg^{2+} (J_{Na}, J_{Cl}, J_{Ca} and J_{Mg}) were measured in six tubules in the absence and presence of an experimentally generated *lumen-to-bath* directed NaCl concentration gradient, active NaCl transport being inhibited by furosemide. Since the paracellular shunt pathway of the mTAL segment is three to four times more permeable to Na^+ than for the Cl^- [7, 14], the generation of a transepithelial *lumen-to-bath* or *bath-to-lumen* directed NaCl concentration gradient in the absence of active NaCl transport should enable us to investigate the relationship between transepithelial Ca^{2+} and Mg^{2+} net fluxes and the transepithelial voltage. A linear relationship between these two parameters would strongly suggest a passive permeability of the mTAL to Ca^{2+} and Mg^{2+}. At zero voltages (symmetrical solutions in lumen and bath), the net fluxes should be zero and at positive or negative voltages (*bath-to-lumen* or *lumen-to-bath* directed NaCl concentration gradient respectively), Ca^{2+} and Mg^{2+} net transport should occur in the direction imposed by the electrical gradient. In the second protocol, J_{Na}, J_{Cl}, J_{Ca} and J_{Mg} and the PD_{te} were measured in ten tubules in the absence and presence of an experimentally generated transepithelial *bath-to-lumen* directed NaCl concentration gradient. In these experiments, active NaCl transport was again inhibited by furosemide.

In the second series, transepithelial ion net fluxes and PD_{te} were measured in nine mTAL segments in the absence and presence of an experimentally generated transepithelial *lumen-to-bath* directed NaCl concentration gradient, to generate high lumen-positive voltages. In this series, transepithelial NaCl net transport was not inhibited by apical furosemide, but reduced to almost zero by using an appropriate hypotonic luminal perfusate [15, 42]. The aim of this maneuver was to increase the transepithelial voltage in the absence of any possible influence of furosemide on the permeability to Ca^{2+} and Mg^{2+} of the paracellular shunt pathway.

In the last experimental series, the effect of ADH (10^{-10} mol.l^{-1}, bath) on the passive permeability to Ca^{2+} and Mg^{2+} of the paracellular shunt pathway was investigated. To look specifically for a possible effect of ADH on this passive permeability, basal and ADH-stimulated NaCl net transport was reduced to almost zero by use of hypotonic luminal perfusate [42]. This kind of experiment should also allow us to exclude any interference of the ADH-mediated voltage increase with an eventual passive divalent cation transport mechanism. Transepithelial ion net fluxes (J_{Na}, J_{Cl}, J_{Ca} and J_{Mg}) and PD_{te} were measured in five isolated mTAL segments.

Under several experimental situations in our study, Ca^{2+} net secretion into the tubular lumen was observed. We tried to identify the origin of this secretion, and more specifically we were interested in exploring whether apical K^+ might be involved in the observed secretory process. To this end, in the fourth experimental series, 25 mmol.l^{-1} Na^+ of the luminal perfusate was replaced by an equimolar quantity of K^+ and transepithelial ion net fluxes (J_{Na}, J_{Cl}, J_{Ca}, J_{Mg}) and PD_{te} measured in five isolated perfused mTAL segments before, during and after substitution of Na^+ by K^+. These experiments were performed in the presence of luminal furosemide (10^{-4} mol.l^{-1}).

Statistics

Data are expressed as mean values ±SEM. The numbers in brackets (*n*) refer to the number of investigated tubules. The Anova test and Bonferroni's multiple comparison test were used to test for statistical significance. A *P*-value of <0.05 was accepted for statistical significance.

Results

Figure 1 shows transepithelial Ca^{2+} and Mg^{2+} net fluxes (J_{Ca}, J_{Mg}), measured in mTAL segments as functions of the transepithelial potential difference (PD_{te}). With symmetrical solutions in lumen and bath and in the presence of luminal furosemide, the PD_{te} was +0.9 ± 0.3 mV (n = 6). In spite of the dissipation of the electrical gradient, Ca^{2+} was significantly secreted into the tubular lumen (J_{Ca}: −0.32 ± 0.08 pmol.min^{-1}mm^{-1}, n = 6). J_{Mg} was not significantly different from zero (J_{Mg}: −0.07 ± 0.09 pmol.min^{-1}.mm^{-1}, n = 16). In the presence of furosemide and a transepithelial *lumen-to-bath* directed NaCl concentration gradient to accentuate the lumen-positive electrical gradient (PD_{te}: +18.7 ± 1.7 mV, n = 6), Ca^{2+} net secretion into the lumen was not significantly modified with respect to that observed under symmetrical conditions, J_{Ca} averaging −0.21 ± 0.06 pmol.min^{-1}mm^{-1} (n = 6). Similar results were obtained for J_{Mg}. The generation of a transepithelial *bath-to-lumen* directed NaCl concentration gradient in the presence of luminal furosemide rendered the PD_{te} lumen-negative (PD_{te}: −15.4 ± 1.5 mV, n = 10), which accentuated the net secretion of Ca^{2+} into the tubular lumen and also elicited a net secretion of Mg^{2+} (J_{Ca}: −0.80 ± 0.14 and J_{Mg}: −0.55 ± 0.15 pmol.min^{-1}.mm^{-1}, n = 10). Thus, in the mouse mTAL, there is no linear relationship between the transepithelial voltage and Ca^{2+} and Mg^{2+} net fluxes. The absence of Ca^{2+} and Mg^{2+} net reabsorption under a high lumen-positive PD_{te} strongly indicates that the passive permeability of the mTAL to divalent cations is very low, in contrast to that to Na^+ and Cl^-. During these experiments J_{Na} and J_{Cl} were simultaneously measured, and Fig. 2 shows that J_{Na} and J_{Cl} were linearily correlated with the PD_{te}. At zero voltage, transepithelial Na^+ and Cl^- net transport rates were zero, at a lumen-positive PD_{te} (PD_{te}: +18.7 ± 1.7 mV, n = 7), Cl^- and Na^+ were secreted into the tubular lumen (J_{Cl}: −97 ± 1.3, J_{Na}: −97 ± 1.3 pmol.min^{-1}.mm^{-1}, n = 7) and at a lumen-negative PD_{te} (PD_{te}: −14.5 ± 1.5, n = 8) Cl^- and Na^+ were reabsorbed (J_{Cl}: +100 ± 21, J_{Na}: +125 ± 25 pmol.min^{-1}.mm^{-1}, n = 8). The rates of NaCl secretion and reabsorption were correlated with the electrochemical gradient of the two ions.

Figure 3 shows the results of an experimental series

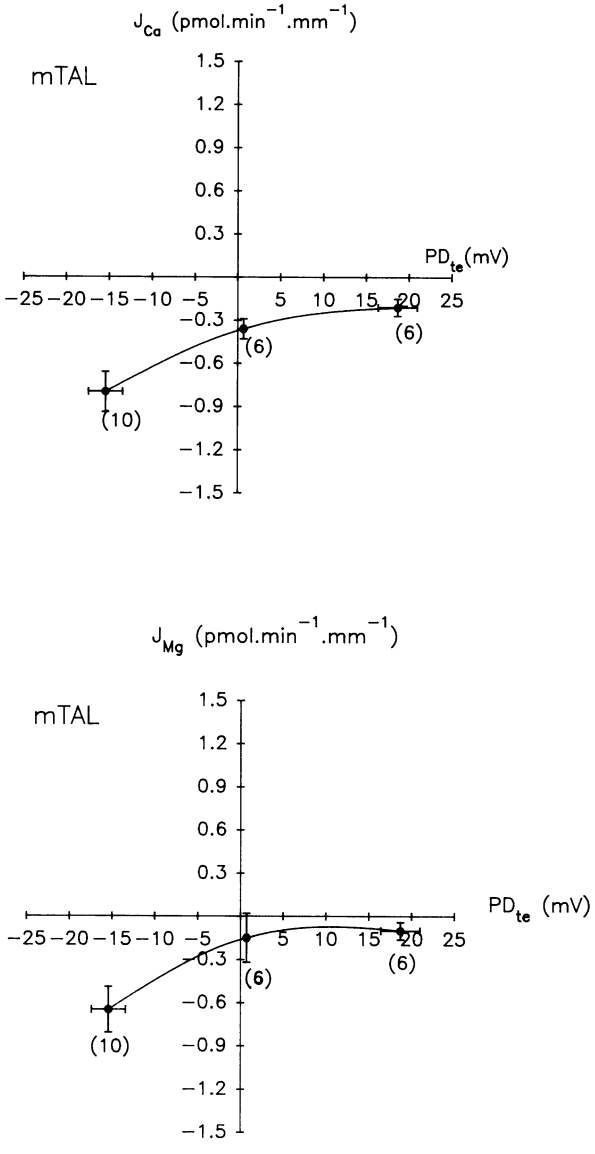

Fig. 1. Relationship between transepithelial Ca^{2+} and Mg^{2+} net fluxes (J_{Ca}, upper graph, J_{Mg}, lower graph) and the transepithelial potential difference (PD_{te}), J_{Ca} and J_{Mg} were measured in mTAL segments in the absence and presence of active NaCl transport (lumen: 10^{-4} mol.l^{-1} furosemide) under three experimental conditions: (i) in the presence of an experimentally generated, transepithelial *bath-to-lumen* directed NaCl gradient, (ii) with symmetrical solutions in lumen and bath, and (iii) in the presence of an experimentally generated *lumen-to-bath* directed NaCl gradient. In all experiments Ca^{2+} and Mg^{2+} concentrations were identical in the lumen and bath solutions. Each data point represents the mean value for PD_{te} and the corresponding flux values, obtained on (*n*) tubules.

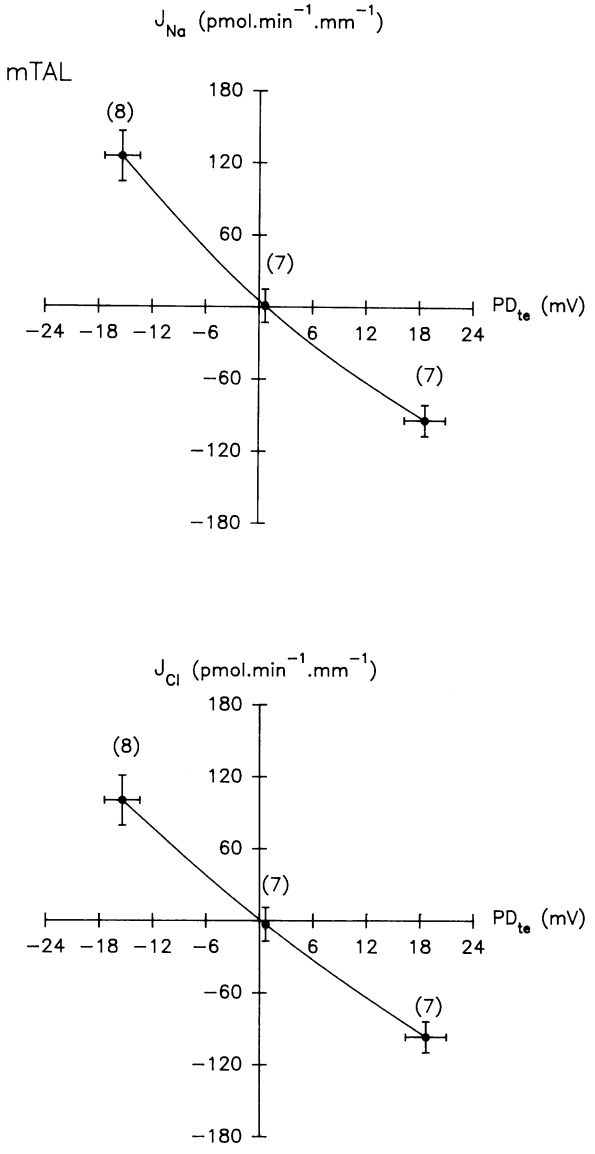

Fig. 2. Relationship between transepithelial Na^+ and Cl^- net fluxes (J_{Na}, upper graph, J_{Cl}, lower graph) and the transepithelial potential difference (PD_{te}), J_{Cl} and J_{Na} were measured in mTAL segments in the absence and presence of active NaCl transport (lumen: 10^{-4} mol.l^{-1} furosemide) under three experimental conditions: (i) in the presence of an experimentally generated, transepithelial *bath-to-lumen* directed NaCl gradient, (ii) with symmetrical solutions in lumen and bath, and (iii) in the presence of an experimentally generated *lumen-to-bath*-directed NaCl gradient. Each data point represents the mean value for PD_{te} and the corresponding flux values, obtained on (*n*) tubules.

in which Ca^{2+} and Mg^{2+} net fluxes were measured in the absence of apical furosemide. The lumen-positive PD_{te} of the mTAL was increased by lowering the NaCl concentration of the luminal fluid with respect to the bath solution. Firstly, under control (symmetrical) conditions, a mean PD_{te} of +8.5 ± 0.8 mV (n = 9), characteristic of mTAL segments was measured, but Ca^{2+} and Mg^{2+} net transport was close to zero, as often reported [1, 8, 9, 22, 41]. After creation of a *lumen-to-bath* directed NaCl concentration gradient, the PD_{te} increased to +23.3 ± 1.2 mV (n = 9). Surprisingly, this increase was paralleled by a significant secretion of Ca^{2+} into the tubular lumen: J_{Ca} increased from −0.01 ± 0.05 (control) to

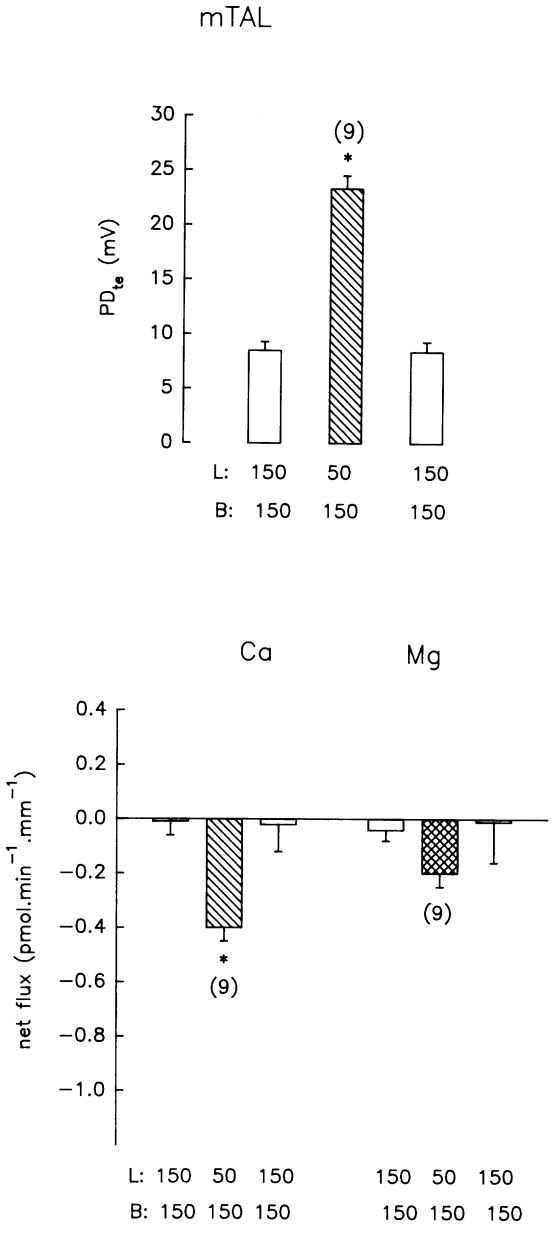

Fig. 3. Effect of a high lumen-positive transepithelial voltage (PD_{te}), experimentally generated by a *bath-to-lumen* directed NaCl gradient, on Ca^{2+} and Mg^{2+} net transport in mouse mTAL segments in the absence of apical furosemide. PD_{te} (upper histogram) and the transepithelial Ca^{2+} and Mg^{2+} net fluxes (J_{Ca}, J_{Mg}, lower histogram) were measured simultaneously under control conditions (lumen, (L), bath (*B*), 150 mM NaCl), in the presence of a hypotonic luminal perfusate (lumen: 50 mM NaCl, bath: 150 mM NaCl) and after return to control conditions (lumen, bath: 150 mM NaCl). Ca^{2+} and Mg^{2+} net movement occurred in the absence of chemical Ca^{2+} and Mg^{2+} gradients. Each column represents the mean value ±SEM for PD_{te} and J_{Ca} and J_{Mg}, measured in (*n*) tubules. *: significantly different from the respective control periods. Note that even in the presence of a high lumen-positive transepithelial voltage no Ca^{2+} and Mg^{2+} net reabsorption occurred and that Ca^{2+} was even secreted into the tubular lumen.

-0.40 ± 0.05 pmol.min^{-1}.mm^{-1}, $n = 9$. With respect to Mg^{2+}, a small but not statistically significant net secretion was noted. The observed phenomena was reversible after return to control conditions.

Figure 4 shows the results of another experimental series in which the lumen-positive PD_{te} of mTAL segments was increased by use of a hypotonic perfusate in the absence of apical furosemide. The PD_{te} was $+29.9 \pm 0.7$ mV ($n = 5$) and as already observed in this study, Ca^{2+} was significantly secreted into the tubular lumen (J_{Ca}: -0.48 ± 0.05 pmol.min^{-1}.mm^{-1}, $n = 5$). In addition, there was a small but significant secretion of Mg^{2+} (J_{Mg}: -0.21 ± 0.05 pmol.min^{-1}.mm^{-1}). Addition of 10^{-10} mol.l^{-1} ADH to the bath had no effect on these Ca^{2+} and Mg^{2+} net movements: both ions were still secreted into the tubular lumen as in the absence of the hormone.

Figure 5 shows the results of experiments in which transepithelial Ca^{2+} and Mg^{2+} net fluxes were measured when the 25 mM Na^+ content of the luminal solution was replaced by 25 mM K^+ in the presence of luminal furosemide. Under these conditions PD_{te} was only moderately changed (from $+0.9 \pm 0.3$ to -1.5 ± 0.2 mV), but this change induced a huge secretion of Ca^{2+} and Mg^{2+} into the tubular lumen, J_{Ca} and J_{Mg} reaching -0.96 ± 0.17 and -0.91 ± 0.24 pmol.min^{-1}.mm^{-1}, $n = 5$, respectively. These secretory processes were reversible after resubstitution of K^+ by Na^+.

Discussion

The present study demonstrates that in the mouse mTAL the transepithelial voltage is not a driving force for Ca^{2+} and Mg^{2+} net movement across the epithelium which strongly suggests that the passive permeability of the mTAL to these divalent cations is very low. This is not the case for monovalent ions such as Na^+ and Cl^-. In previous studies [19, 32, 39] it had been established that in the mouse mTAL Na^+ and Cl^- are transported both, passively and actively. The present study demonstrated that during active NaCl transport inhibition by luminal furosemide, the experimentally generated transepithelial voltage directly governed the direction and amplitude of transepithelial Na^+ and Cl^- net movement. Simultaneously, in the same tubules a lumen-positive PD_{te} was unable to create any transepithelial Ca^{2+} and Mg^{2+} net reabsorption. In this nephron segment, the paracellular shunt pathway therefore displays a marked ionic selectivity. On the other hand, a lumen-negative PD_{te} induced Ca^{2+} and Mg^{2+} net secretion into the tubular lumen. Such secretory processes might have resulted from passive movements through the paracellular pathway. If this hypothesis were true, then a *bath-to-lumen* directed NaCl concentration gradient, generating a lumen-positive PD_{te}, should have produced Ca^{2+} and Mg^{2+} net reabsorption which, however, was not observed.

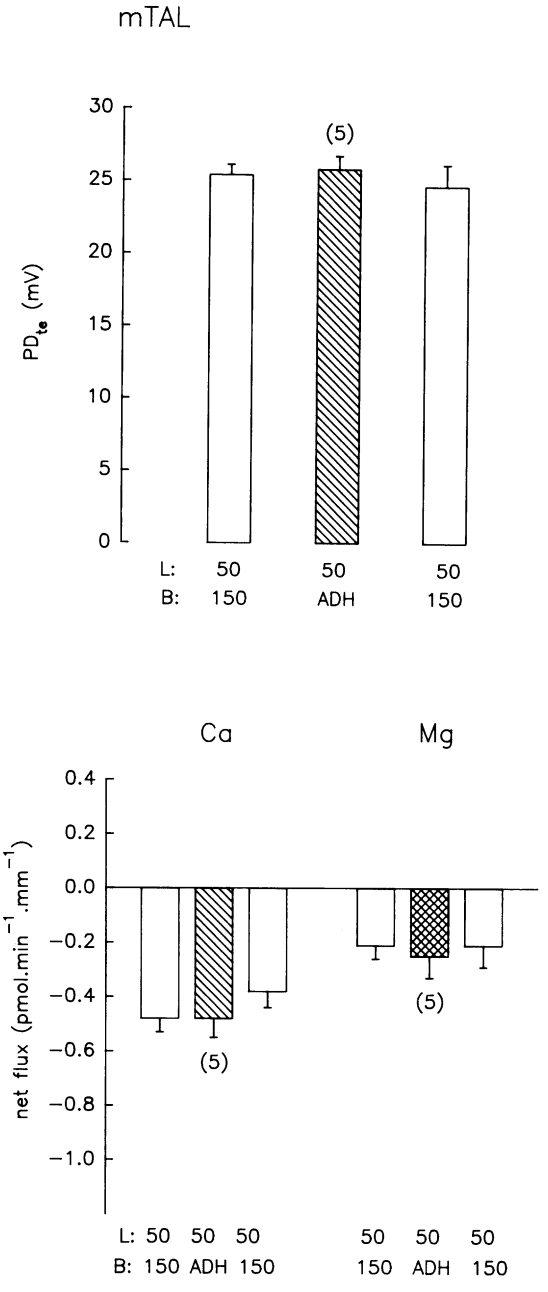

Fig. 4. Effect of ADH (10^{-10} mol.l^{-1}, bath) on Ca^{2+} and Mg^{2+} net transport in mouse mTAL segments, when active NaCl transport was experimentally reduced by a *bath-to-lumen* directed NaCl gradient (lumen (L): 50 mM NaCl, bath (*B*): 150 mM NaCl) in the absence of apical furosemide. PD_{te} (upper histogram) and the transepithelial Ca^{2+} and Mg^{2+} net fluxes (J_{Ca}, J_{Mg}, lower histogram) were measured simultaneously in the presence of a hypotonic luminal perfusate (lumen: 50 mM NaCl, bath: 150 mM NaCl), with ADH absent (open bars) or present (hatched and cross-hatched bars) in the peritubular solutions. The observed Ca^{2+} and Mg^{2+} net secretions were not modified by ADH and occurred in the absence of chemical Ca^{2+} and Mg^{2+} gradients. Each column represents the mean value ±SEM for PD_{te} and J_{Ca} and J_{Mg}, measured in (*n*) tubules.

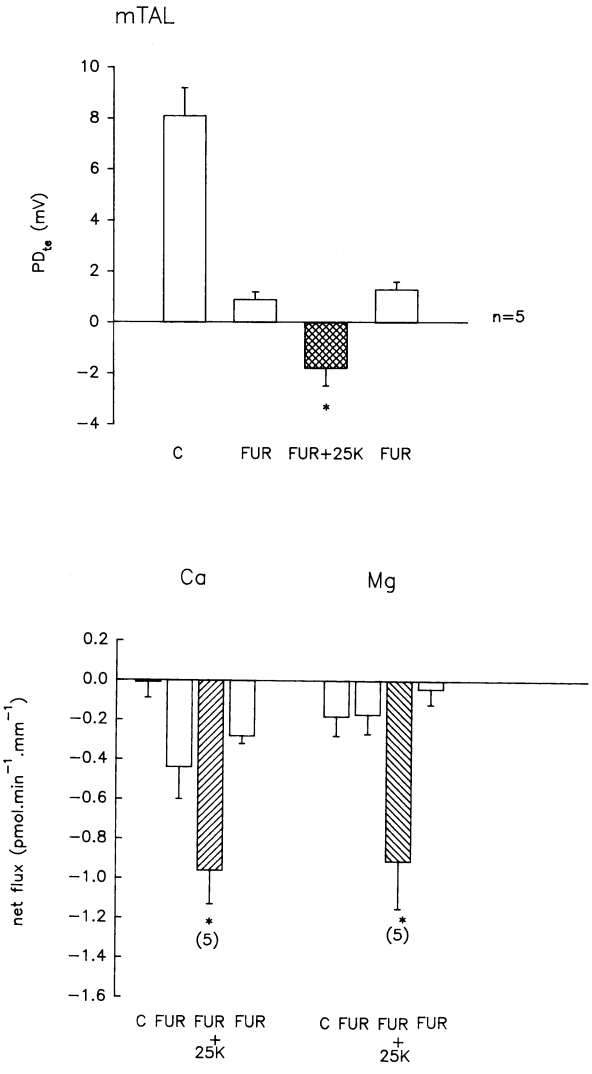

Fig. 5. Effect of equimolar substitution of Na^+ by K^+ in the luminal perfusate in the absence of active NaCl transport (lumen: 10^{-4} mol.l^{-1} furosemide) on Ca^{2+} and Mg^{2+} net transport in mouse mTAL segments. The transepithelial voltage (PD_{te}, upper histogram) and the transepithelial Ca^{2+} and Mg^{2+} net fluxes (J_{Ca}, and J_{Mg}, lower histogram) were measured simultaneously under four experimental conditions: (i) under control conditions (*C*, lumen and bath: 150 mM NaCl); (ii) in the presence of luminal furosemide (FUR, lumen and bath: 150 mM NaCl); (iii) after substitution of 25 mM NaCl by 25 mM KCl in the luminal perfusate (FUR + 25 KCl); iv) in the presence of luminal furosemide (FUR, lumen and bath: 150 mM NaCl). Each column represents the mean value ±SEM for PD_{te} and J_{Ca} and J_{Mg}, measured in (*n*) tubules. *: significantly different from the respective control periods.

In the presence of luminal furosemide, the mouse mTAL secretes significant amounts of Ca^{2+}, a process already reported for the mouse cTAL [6, 42]. Since in the presence of furosemide transepithelial driving forces for Ca^{2+} net movement are negligible, it is very likely that some Ca^{2+} of cellular origin is added to the tubular fluid. More importantly, Ca^{2+} net secretion in the mTAL

even occurred when the PD_{te} was markedly lumen-positive.

Opposite observations were made for the cTAL segment of the same species [6, 42], in which a lumen-positive PD_{te}-generated transepithelial Ca^{2+} and Mg^{2+} reabsorption. For the cTAL segment it was furthermore shown [6] that transepithelial Ca^{2+} and Mg^{2+} transport was linearly correlated with the transepithelial voltage, so that Ca^{2+} and Mg^{2+} secretion turned into reabsorption and *vice-versa,* depending on whether the transepithelial voltage was experimentally clamped to positive or to negative values. We had therefore suggested that in the cTAL transepithelial Ca^{2+} and Mg^{2+} transport is a passive, paracellular process. Since no linear relationship exists between the transepithelial voltage and transepithelial divalent net fluxes in the mouse mTAL, we have to conclude that the passive permeability of the mTAL for divalent cations is very low. The tubular secretion of Ca^{2+} and Mg^{2+} observed under different experimental conditions in this nephron segment has therefore to be of cellular origin.

ADH, a hormone known to stimulate transepithelial NaCl reabsorption in both the cortical and medullary portion of the thick ascending limb [18, 20, 32, 33, 39, 41], had no effect on transepithelial Ca^{2+} and Mg^{2+} diffusion in the mouse mTAL. The action of ADH was tested in the presence of a transepithelial *bath-to-lumen* directed NaCl concentration gradient, a maneuver previously shown to enhance the stimulatory effect of PTH and insulin on transepithelial Ca^{2+} and Mg^{2+} reabsorption in the mouse cTAL, without, however, affecting that of NaCl [22, 42]. The present results strongly suggest that in the mTAL, ADH does not render the epithelium permeable for Ca^{2+} and Mg^{2+}. This even holds true in the presence of a hypotonic luminal fluid which generated a high lumen-positive transepithelial voltage, supposed eventually to induce Ca^{2+} and Mg^{2+} net reabsorption in the mTAL.

In the mTAL, a consistent and sustained secretion of Ca^{2+} and Mg^{2+} into the tubular lumen was induced either by lowering the luminal NaCl concentration or by raising that of K^+. Lowering the luminal NaCl concentration is known to lead to cellular hyperpolarization and a lowered transepithelial voltage [13], while increase in luminal K^+ concentration results in a depolarization of the apical membrane voltage [17]. In the present study, luminal K^+ concentration was increased in the presence of furosemide which turned off the apical $Na^+2Cl^-K^+$ cotransporter and thus the K^+ recycling across the apical membrane. Both, a decrease in luminal NaCl concentration and an increase of that of K^+ induced sustained Ca^{2+} and Mg^{2+} net secretion towards the tubular lumen, although these maneuvers resulted in opposite effects on the membrane potentials. This would suggest that the observed secretions are the outcome of multifactorial processes which are primarily or secondarily sensitive to the membrane potential or to intracellular events.

What might be the cellular mechanisms involved in the observed luminal net secretion of Ca^{2+} and Mg^{2+}? The secretion of Ca^{2+} and Mg^{2+} clearly occurred against an electrochemical gradient. A priori, two apical transport components could be involved: either a (Ca^{2+}-Mg^{2+})-ATPase or a Na^+-(Ca^{2+}-Mg^{2+})-exchanger, the latter being energized by the electrochemical gradient for Na^+ entry into the cell. The presence of a Na^+-(Ca^{2+}-Mg^{2+})-exchanger seems unlikely since neither immunohistochemical techniques nor molecular biological approaches have been able to detect this transport protein in the thick ascending limb [27,28]. Furthermore, lowering the NaCl concentration of the luminal perfusate relative to that of the peritubular solution would have reduced Ca^{2+} or Mg^{2+} net secretion towards the tubular lumen, had such a Na^+-(Ca^{2+}-Mg^{2+})-exchanger existed. In fact, we observed an increase in tubular Ca^{2+} and Mg^{2+} secretion. The presence of a Ca^{2+}-ATPase and a Mg^{2+}-ATPase in the apical plasma membrane of the mTAL could be responsible for the observed Ca^{2+} and Mg^{2+} secretion. The presence of a plasma membrane Ca^{2+}-ATPase has largely been described for the kidney cortex [10, 12, 36, 38]. A Mg^{2+}-ATPase could also be present, as that one described for the human erythrocyte [2, 25] and the distal tubule of the human kidney [2].

The presence of such a Ca^{2+}- and/or Mg^{2+}-ATPase in the luminal membrane of the TAL has, however, still to be demonstrated. In vivo, the thick ascending limb reabsorbs Ca^{2+} and Mg^{2+}. Following diuresis induced by loop diuretics, Ca^{2+} and Mg^{2+} as well as Na^+, Cl^- and K^+ are no longer reabsorbed in the TAL, leading with respect to Ca^{2+} and Mg^{2+} to calciuria and magnesiuria. The additional small secretion of Ca^{2+} and Mg^{2+} observed in vitro under furosemide perfusion in the mTAL (*present study*) and cTAL segment [6, 42] may not be important in terms of Ca^{2+} and Mg^{2+} balance when compared to the large loss of both cations due to the furosemide-induced inhibition of Ca^{2+} and Mg^{2+} reabsorption, but this secretion might be important for cell Ca^{2+} and Mg^{2+} balance. The large secretion of Ca^{2+} and Mg^{2+} observed in the in vitro perfused mTAL segment perfused with hypotonic solutions, does not reflect the physiological behavior of the mTAL. However, the surrounding of this nephron segment is hypertonic and we actually do not know whether a hypertonic medium affects Ca^{2+} and Mg^{2+} transport in the mTAL. Even if this nephron segment secretes Ca^{2+} and Mg^{2+} under particular conditions, a large portion of Ca^{2+} and Mg^{2+} is supposed to be reabsorbed in the cortical part of the TAL, so that over all Ca^{2+} and Mg^{2+} reabsorption will be observed in the thick ascending limb in vivo.

In conclusion, we have shown in the present study that the mTAL segment of the mouse is characterized by

a very low permeability for Ca^{2+} and Mg^{2+}. The mTAL and very possibly also the cTAL are sites along the nephron at which these cations can be secreted against their electrochemical gradient from the intracellular domain towards the tubular lumen. Further studies to establish the nature of these mechanisms are clearly warranted.

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