

Characteristics of urea transport of cells derived from rabbit thick ascending limb of Henle's loop

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Background. The thick ascending limb of Henle's loop (TALH) is thought to be involved in the regulation of the renal urea gradient.

Methods. We have characterized the uptake of urea (oil density centrifugation and 2-compartment-culture) and volume regulation (impedance measurement) in highly differentiated cells derived from rabbit outer medulla.

Results. TALH cells exposed to 600 mOsm/liter (300 mM urea) shrunk to $72 \pm 5\%$ of the isoosmotic volume. Due to a regulatory volume increase (RVI), the cell volume was almost completely regained at $92 \pm 6\%$ after five minutes. The uptake of ^{14}C -urea in the presence of urea concentrations up to 600 mM did not show any saturation. In the presence of phloretin the urea uptake decreased to $69 \pm 14\%$. The transport was sodium and chloride independent. Changing the membrane potential caused an increase of regulatory volume increase and urea uptake. Hyperosmolarity induced by sucrose (300 mM) and NaCl (150 mM) caused a decrease of urea uptake to $70 \pm 14\%$ and $53 \pm 11\%$, respectively. The permeability coefficient (P) in a two compartment culture was $P = 1.7 \cdot 10^{-6} \pm 0.39 \cdot 10^{-6}$ cm/second, suggesting a relatively low permeability.

Conclusion. Due to the low permeability, it seems impossible to achieve a physiologically significant participation of the TALH in the urea circulation within the nephron. However, the results of this study provides significant hints about the existence of a specific urea transport mechanism that enables the cell to adapt rapidly to different osmolarities.

Urea plays an important role in the osmotic regulation of the kidney. To create a highly concentrated urine, a high concentration of urea is necessary in the interstitium of the inner medulla. Various mechanisms are involved to achieve and maintain this rapid transport of urea across the terminal part of the inner medullary collecting duct (IMCD), which is crucial in order to establish and maintain the inner medullary urea gradient. There are several urea transports (UT), such as the vasopressin-sensitive UT1 [1], involved. In addition, a secondary active sodium-dependent transport [2] and a urea-transporting water channel of the aquaporin family [3] have been observed in rat.

Urea, which leaves the inner medulla via the ascending vasa recta, is partially recycled by transfer to adjacent

descending limbs of Henle's loop [4]. The high permeability for urea of this part of the loop may be due to UT2 [5].

A special urea transport was found in the descending vasa recta [6, 7], which suggests counter current exchange of urea between the ascending and the descending vasa recta. Recently this transport was identified as UT3 [8].

The loop of Henle has been proposed to be an important factor to maintain the urea gradient. Urea permeability is relatively high in the straight proximal tubule [9], indicating the possibility of an active transport system [10]. The high concentration of urea in the inner medulla causes a urea stream into the thin descending and the beginning of the thin ascending limb. The decrease of the interstitial urea concentration towards the cortex leads to an inversion of the urea concentration profile, and thereby to urea absorption out of the thin ascending limb [4].

The thick ascending limb of Henle's loop (TALH) was supposed to be impermeable for urea. Rocha and Kokko discovered low permeability coefficients concerning the inner stripe of the outer medullary ascending limb [11]. However, at least the cortical part of the rabbit thick ascending limb is relatively highly permeable [9]. Therefore, urea absorption out of the cortical ascending limb and secretion in the descending limb appears to be possible. In fact this mechanism may be necessary in order to build up a urea gradient together with a high amount of urea absorption in the IMCD. Therefore, it appears questionable whether any parts of the medullary thick ascending limb have a share in the urea circulation.

The purpose of this study was to characterize urea uptake and volume regulation under conditions of urea-induced hyperosmolarity with cells from the thick ascending limb, derived from the outer medulla. Our results indicate the existence of a specific urea transport mechanism in rabbit TALH that is probably necessary for a proper volume regulation.

METHODS

Cell culture

The cells used for this study were a SV40 transfected, highly differentiated established cell line derived from the

Key words: renal urea gradient, isoosmotic volume, hyperosmolarity, permeability, interstitium, TALH.

Received for publication July 9, 1997
and in revised form February 11, 1998
Accepted for publication February 12, 1998

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outer medulla of rabbits, showing typical characteristics of cells of the thick ascending limb of Henle's loop (TALH) [12, 13].

The cells were cultured in 300 mOsm/liter Dulbecco's modified Eagle's medium with a 10% supplement of fetal calf serum at 37°C and 5% CO₂. Within three to five days after dissemination, a dense cell monolayer occurred in the culture bottles. For all experiments the cells were detached from the culture bottles by trypsinization and were resuspended in HEPES-Ringer buffer (118 mM NaCl, 16 mM H-HEPES, 16 mM Na-HEPES, 3.2 mM KCl, 2.5 mM CaCl₂, 5 mM glucose, 10 mM pyruvate, 1.8 mM MgSO₄, 1.8 mM KH₂PO₄, 2 mM Na-acetate; pH 7.4, 300 mOsm/liter).

For some experiments cells adapted to 600 mOsm/liter were used. Osmolarity was adjusted by the appropriate addition of 150 mM NaCl or 300 mM sucrose, respectively. The adapted cells were cultured through more than 10 passages in the increased osmolarity before using them in the experiment. The cells underwent 40 to 80 passages in total.

For the two-compartment-culture, permeable cell culture filter inserts (0.4 μm pore size, 4.3 cm² surface; Falcon, Heidelberg, Germany) were placed in Multiwell tissue culture plates (Falcon). The cells formed a polarized tight monolayer within two to four days. The detachment of the cells of the filter inserts was effected as described above.

Characterization of urea transport

The cells were pre-equilibrated with ³H₂O (Du Pont de Nemours, Bad Homburg, Germany), which readily enters the cell. It was used as a marker for the cell's water content. The incubation was started by the addition of ¹⁴C-urea (Amersham, Braunschweig, Germany). Typically, if not stated otherwise, the cells were separated after 50 seconds from the ³H₂O and ¹⁴C-urea containing extracellular medium by 15 seconds centrifugation (at 14000 g; Beckmann Microfuge E) of the aliquot through a silicon oil mix (AR20 and AR200; Wacker Chemie, Munich, Germany). Because of the varying osmolarities of the tested aliquots different oil mix ratios were needed to reach the appropriate density of the oil. The following AR20:AR200 ratios were used: 1:1.5, 1:2, 1:3.5 and 1:4. The activities of the isotopes were measured via liquid scintillation counting (Wallac, Turku, Finland) after dissolving the pellets in 1 M NaOH solution and mixing this with Ultima Gold (Packard, Groningen, Netherlands) [14]. The portion of the extracellular medium, which was adherent despite the spinning through the oil mix, was determined in a simultaneous parallel test. For this purpose, cells were incubated with ¹⁴C-sucrose (marker for extracellular space [15]; Du Pont de Nemours) instead of ¹⁴C-urea. The extracellular portion in relation to the cellular volume was about 27%.

The saturation of urea uptake was determined at extracellular urea concentrations of 5, 100, 200, 300, 400, 500

and 600 mM. Urea uptake was determined at 37°C and at 4°C.

To determine the influence of hyperosmolar conditions on urea uptake, TALH cells adapted to 600 mOsm/liter (with the addition of 150 mM NaCl or 300 mM sucrose to the DMEM) were used.

To test sodium and chloride dependency, NaCl was replaced by N-methyl-D-glucosamine, Na-HEPES by H-HEPES and Na-acetate by K-acetate, Cl⁻ was replaced by using NaNO₃ instead of NaCl, exchange of KCl for K-acetate and replacing CaCl₂ by Ca-sulfate.

The influence of the cell's membrane potential on the urea uptake was examined via raising the potassium-concentration by 50 mM or via the presence of 90 μM of the potassium ionophor valinomycin, respectively.

To check whether urea uptake could be inhibited, the influence of the structure analogs thio-methylurea, N-methylurea and acetamide with concentrations of 50 mM each was tested. The osmolarity in the controls was adjusted by the addition of 50 mM sucrose.

The effects of 0.5 mM phloretin (Sigma Chemie, Deisenhofen, Germany) and 0.5 mM 4-(chloromercuri)phenyl-sulfonic acid (pCMBS; Sigma) were examined as well.

Determination of the volume regulation of TALH cells

TALH cells were pre-incubated with HEPES-Ringer buffer for 30 minutes. After trypsinization, the detached cells were resuspended in the HEPES-Ringer buffer and incubated for five minutes. Then the cell suspension was diluted 1:10 with 600 mOsm/liter urea-HEPES-Ringer buffer with an additive of the substance to be tested. The cell volume was determined by impedance measurement with a Coulter Counter ZM plus Coulter Channelizer 256 after 20, 40, 60, 90, 120, 300 and 600 seconds.

To check the influence of 0.1 mM Br-cAMP (8-bromo-adenosine 3': 5'-cyclic-monophosphate; Sigma) cells were pre-incubated for five minutes.

The effect of the membrane potential on volume regulation were determined as described above.

Furthermore, the effects on the volume regulation of 5 mM BaCl₂ (Sigma), 1 mM 4-acetamido-4'-iothio-cyanato-stilbene-2,2'-disulfonic acid (SITS; Sigma), 1 mM amiloride (Sigma), 10 mM ouabain (Fluka, Heidelberg, Germany), 0.5 mM phloretin (Sigma) and 0.1 mM furosemide (Sigma) were examined.

Polarity of the urea uptake via a two-compartment culture

First, the cell monolayer was incubated for one minute from the luminal side with HEPES-Ringer-buffer containing 5 mM ¹⁴C-urea and ³H-inulin (Amersham). The basolateral compartment contained urea-free HEPES-Ringer buffer. After the elapsed incubation period the buffer was extracted. Then, in order to remove the remaining adherent buffer, the filter inserts were washed four times in

Table 1. Urea uptake via the luminal membrane at 37°C and 4°C in absolute and percental numbers

	37°C	37°C-4°C	4°C
mmol/hr/g protein	0.27 ± 0.073	0.245	0.024 ± 0.024
%	100 ± 27.2	91	9 ± 8.8

The uptake at 37°C is equivalent to 100% ($N = 13-21$).

ice-cold 900 mOsm/liter urea-HEPES-Ringer buffer. As indicated by previous experiments, this method removes the buffer sufficiently and minimizes ^{14}C -urea losses out of the cells. After drying the cells were detached from the filter with 1 ml 1 M NaOH. One aliquot was used for scintillation counting and one for protein determination. The portion of the adherent extracellular medium was determined via the activity of ^3H -inulin and was 0.02%.

To determine which part of the urea uptake was caused by unspecific transport, urea uptake was measured in parallel at 37°C and 4°C (Table 1). Provided that under conditions of 37°C the urea uptake consisted of unspecific diffusion and a further, specific transport mechanism, which was responsible for the greater transport capacity at 37°C, we believed that the difference in the data measured under 37°C and 4°C would show the transport capacity of this specific transport.

To discover possible leaks in the cell monolayer, the ^3H -inulin concentration was measured at the end of the incubation period. The magnitude of the error shown by ^3H -inulin was $0.031 \pm 0.011\%$. Each value over 0.05% led to the exclusion of the experiment.

The portion of radioactively labeled buffer, which passed through the filter and the cell monolayer under the conditions at 4°C, was used to indicate the paracellular transport rate. It was determined by measuring the ^{14}C -urea concentration in the original ^{14}C -urea-free buffer and came to $0.167\% \pm 0.032\%$ ($N = 39$).

Determination of the urea permeability coefficient

The permeability coefficient (P) can be calculated according to

$$dm/dt = P \cdot A \cdot (C_1 - C_2) \quad (\text{Eq. 1})$$

on the basis of the measured total transport rate, where A is the surface area involved in the molecule exchange, corresponding to the surface of the monolayer, and $C_1 - C_2$ is the concentration difference between the urea containing compartment and the urea-free intracellular fluid and basolateral compartment.

Protein determination

The protein concentration was determined according to Lowry [16] in a spectrophotometer (Perkin Elmer Lambda 2; Perkin Elmer, Stuttgart, Germany) at 750 nm. Various concentrations of bovine serum albumin were used as standards.

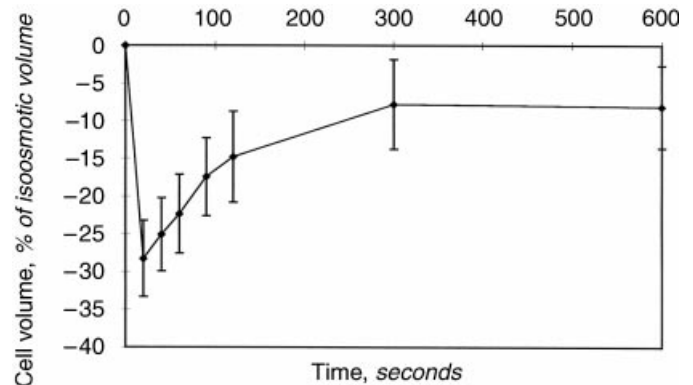


Fig. 1. TALH cells were exposed to 600 mOsm/liter conditions (by addition of 300 mM urea). The change of the cell volume of TALH cells under hyperosmotic conditions over the time is shown. The cell volume under isosmotic conditions is equivalent to 0% (1599 fl). The cell volume under hyperosmotic conditions differs at each measuring time from the control ($N = 19$, $P < 0.0001$).

Statistical analysis

For statistical analysis the single-sample t -test was used, and the difference was considered significant at $P < 0.05$. Mean values with their respective SD are given throughout the paper.

Materials

All chemical reagents were of the highest purity commercially available.

RESULTS

Determination of the volume regulation of TALH cells

Raising the osmolarity from 300 to 600 mOsm/liter, the cell volume decreased rapidly within 20 seconds from 1599 ± 148 fl to 1151 ± 96 fl ($N = 18$, $P < 0.0001$), equivalent to a cell shrinkage to $72 \pm 5\%$ of the control volume. Afterwards the cells showed a typical regulatory volume increase (RVI). Within five minutes, the volume increased again to $92 \pm 6\%$ ($N = 19$), which was maintained until the end of the measurement (Fig. 1). The cells did not reach 100% of the control volume completely within 10 minutes ($P < 0.0001$).

A total of 0.1 mM Br-cAMP had no measurable effect on volume regulation and the RVI of the TALH cells.

Changing the membrane potential by increasing the potassium concentration up to 50 mM enhanced the RVI under hyperosmolar conditions (600 mOsm/liter, 300 mM urea), and the cells regained a larger volume (Fig. 2).

Neither BaCl_2 , nor furosemide, SITS, amiloride, phlor- etin or ouabain influenced the RVI (data not shown).

Characterization of urea transport

Investigation of the characteristics of urea transport showed that uptake reached a steady-state at a level of 93%

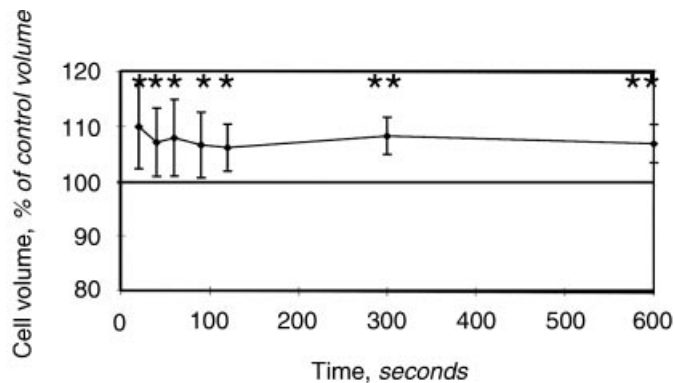


Fig. 2. Influence of a lowered membrane potential (by addition of 50 mM potassium) on the volume regulation of TALH cells. The experiment was executed under hyperosmotic conditions induced by 300 mM urea. Figure 2 shows the volume in percent related to the volume of TALH cells under hyperosmotic (300 mM urea), normokalemic conditions. The cell volume is significantly higher than the volume of the control incubation. * $P < 0.05$; ** $P < 0.01$ ($N = 5$).

$\pm 9\%$ ($N = 4$) of the extracellular urea concentration within 10 minutes of incubation (Fig. 3).

The intracellular concentration reached 1.95 ± 0.34 mmol urea per liter cell water ($N = 21$) after 50 seconds of incubation with 5 mM urea at 37°C . Uptake was $34\% \pm 7\%$ ($N = 7$) of this value at 4°C .

Investigations regarding the kinetic of urea uptake showed no sign of saturation of the transport process up to a concentration of 600 mM (Fig. 4).

Hyperosmolarity, induced by an increased NaCl or sucrose concentration reduced the urea uptake significantly (Table 2).

Changing the membrane potential led to an increase of urea uptake, correspondingly, $90 \mu\text{M}$ valinomycin revealed a significant increase of urea uptake (Table 2).

Urea uptake was not changed by sodium-free conditions, nor by chloride-free incubation (Table 2).

The most effective inhibition of urea uptake was induced by phloretin. However, there was no significant difference between the extent of inhibition caused by phloretin, or by pCMBS (Fig. 5), or by the tested structure analogs, respectively (Fig. 6).

Urea uptake measurement via a two-compartment culture

According to the two-compartment-culture uptake values, the luminal cell membrane seemed to be nearly impermeable for urea under conditions of 4°C (Table 1).

Under conditions of 37°C there was a strong increase of the total transport rate. The proportion of the unspecific transport over the total transport rate was approximately one tenth (Table 1).

Urea permeability coefficient

The permeability coefficient of the luminal membrane was $P = 1.71 \cdot 10^{-6} \pm 0.38 \cdot 10^{-6}$ cm/second. As a control of

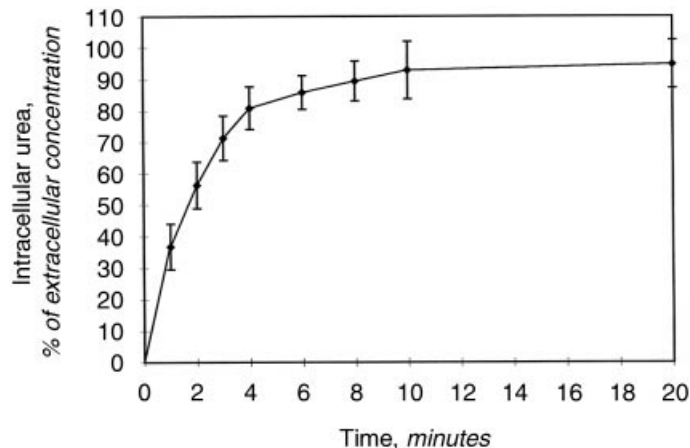


Fig. 3. Urea uptake of TALH cells over the time. Extracellular urea concentration 0.04 mM. The intracellular urea concentration during the incubation period is shown related to the extracellular urea concentration. ($N = 4$).

this result, the average urea permeability of TALH cell membrane can be estimated on the basis of the results of the oil density centrifugation experiments and cell volume measurement. The intracellular urea concentration of TALH cells was 90 ± 10 mmol/liter cell-water ($N = 8$) after 50 seconds of incubation with 300 mM urea. The cell volume under these conditions was 1193 fl. On the basis of these results, according to Eq. 1, a permeability coefficient of $1.32 \cdot 10^{-6}$ cm/second can be calculated. This value corresponds well with the result of the two-compartment culture experiments.

DISCUSSION

The rapid regulatory volume increase of the TALH cells under conditions of 600 mOsm/liter (300 mM urea) raises the question of the existence of specific transport mechanisms for urea and the conditions under which this is accomplished.

The cells show a typical RVI, which leads to an almost complete regeneration of cell volume to the size of cells under isoosmotic conditions. This rapid type of RVI occurred only using urea as an extracellular osmolyte. Using NaCl or sucrose, no RVI could be observed [17].

The lack of complete volume regeneration within the 10 minute testing period may be due to the incomplete equilibration of urea or organic osmolytes.

Urea permeability in the TALH

In the inner stripe of the outer medulla, urea permeability of the TALH amounts to $0.6 \cdot 10^{-5}$ cm/second [11]. This amount is considerably lower than the one in the cortical section of $2 \cdot 10^{-5}$ cm/second [9], which allows physiologically significant urea absorption. Urea permeability in the outer stripe of the ascending limb of the Henle's loop of rabbits has not been described yet. The permeability value

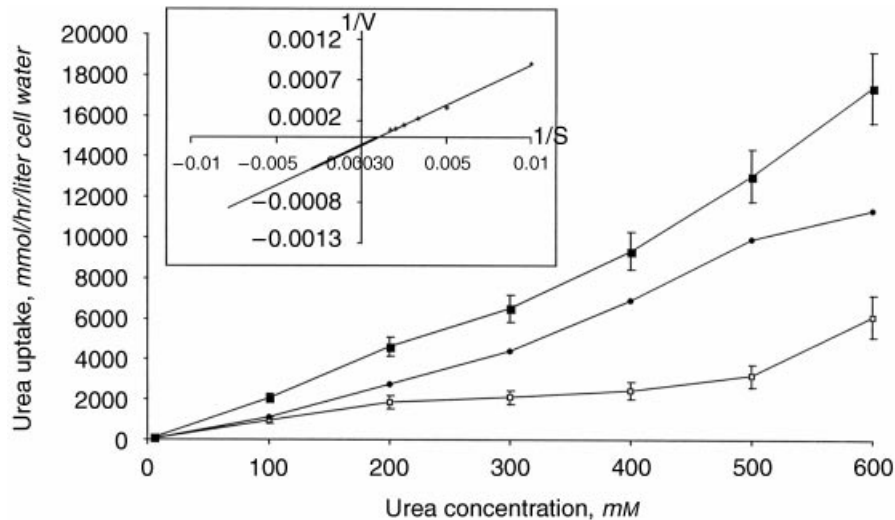


Fig. 4. Kinetic of the urea uptake. The urea uptake of TALH cells is shown related to the extracellular urea concentration (●). The urea uptake measurement under 37°C conditions (■) was corrected by the unspecific transport, measured at the respective extracellular urea concentration under 4°C conditions (□). Window shows Lineweaver-Burk graph. X-axis: $1/S$ [mM^{-1}]; Y-axis: $1/V$ [$(\text{mmol/hr/liter cell water})^{-1}$]

Table 2. Influence of a changed membrane potential (increased KCl or valinomycin incubation), hyperosmolarity (600 mOsm/liter), Na-free and Cl-free conditions on urea uptake in absolute and percental numbers (in relation to control uptake)

	uptake (mmol/h/l cell water)	Relative uptake %	N	P
Hyperosmolarity (NaCl)	87 ± 19	53 ± 11	4	<0.001
Control	165 ± 5			
Hyperosmolarity (sucrose)	87 ± 26	70 ± 14	4	<0.005
Control	123 ± 22			
KCl 50 mM	215 ± 26	168 ± 18	4	<0.005
Control	129 ± 20			
Valinomycin 90 μM	158 ± 16	110 ± 7	8	<0.05
Control	150 ± 17			
Na-free	123 ± 18	98 ± 21	4	NS
Control	129 ± 20			
Cl-free	96 ± 8	77 ± 17	4	NS
Control	129 ± 20			

of the apical membrane measured in this study is $1.7 \cdot 10^{-6} \pm 0.39 \cdot 10^{-6}$ cm/second. Thus, it is in the same range as the values described for the inner stripe. In TALH of rats, Knepper [18] found a value of $1.4 \cdot 10^{-5}$ cm/second for the outer stripe, which does not differ significantly from the value of the cortical ascending limb determined in the same study ($1.5 \cdot 10^{-5}$ cm/second). This difference in the size of one order of magnitude need not to be a contradiction, it might be due to species differences, experimental methods or to the origin of the cells used.

Characteristics of urea transport in TALH cells

Urea uptake was reduced under hyperosmolarity, induced by NaCl or sucrose. The used cells were adapted to the hyperosmolar conditions, therefore no volume shift or cell shrinkage should occur. There are no significant differences in the volume of cells adapted to 600 mOsm/liter NaCl or sucrose and 300 mOsm/liter conditions [17]. Thus, it seems likely that hyperosmolar conditions *per se* inhibited

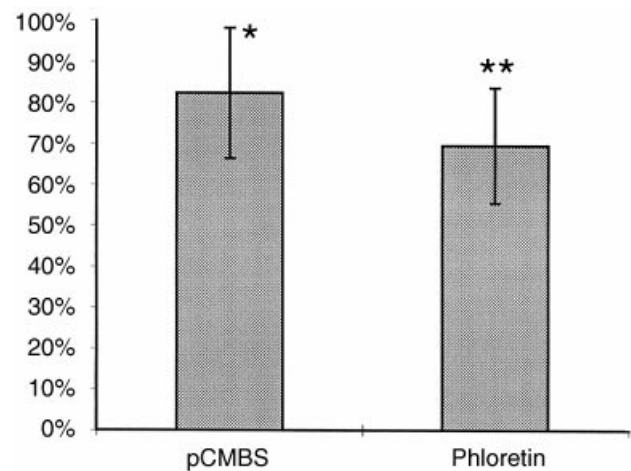


Fig. 5. Inhibition of the urea uptake. Urea uptake in the presence of phloretin (0.5 mM) or pCMBS (0.5 mM) was measured. Osmolarity 350 mM, osmolarity adjustment via sucrose. The urea uptake of the control incubation (50 mM sucrose) is equivalent to 100% (143 mmol/hr/liter cell water ± 23 mmol/hr/liter cell water). * $P < 0.01$; ** $P < 0.0005$ ($N = 8$ to 9).

urea uptake in the TALH cells. A similar effect of hyperosmolarity was described for the $\text{Na}^+ - \text{K}^+ - 2\text{Cl}^-$ cotransport of mouse medullary TALH [19]. A stimulative effect of hyperosmolarity on urea transport in the rat IMCD was found, as well [20, 21]. Thus, osmolarity seems to be an important factor in regulating transport processes and hyperosmolarity seems to inhibit urea uptake of TALH-cells.

The presence of 90 μM valinomycin raises urea absorption, which is probably due to the membrane potential changed by valinomycin. This suggestion is supported by the intense increase of urea uptake and the increased RVI occurring when increasing the extracellular potassium concentration by 50 mM. These differences are due to a significantly lesser negatively charged membrane potential

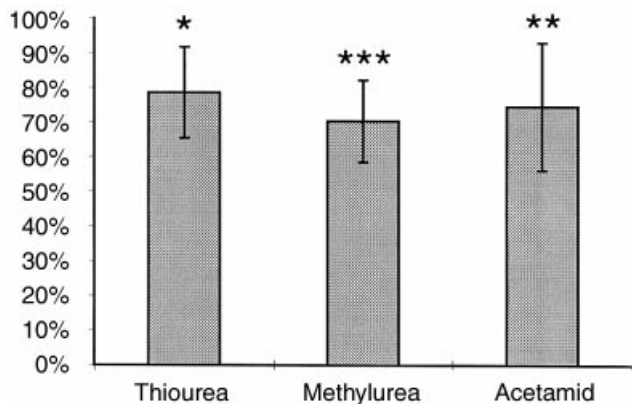


Fig. 6. Reduction of urea uptake in presence of different structure analogs (50 mM, respectively). Osmolarity 350 mOsm/liter, osmolarity adjustment of the control incubation via sucrose, incubation period 50 seconds, 37°C. The urea uptake of the control incubation is equivalent to 100% (143 mmol/hr/liter cell water \pm 23 mmol/hr/liter cell water). * P < 0.01; ** P < 0.001; *** P < 0.0005 (N = 6 to 10).

E_m because of the high extracellular potassium concentration. The parallel absorption behavior under the conditions of valinomycin and increased potassium-concentration, respectively, indicates that the negative membrane potential of the cell is also being reduced by valinomycin. The higher urea uptake under these conditions indicates a transport process that is linked to an ion, such as an anion symport or a cation antiport.

We cannot prove this thesis. It seems rather unlikely that this ion is potassium. A barium-sensitive potassium-channel has been described for the apical membrane of cells from the TALH. This channel plays an important role in the potassium reabsorption [12, 22, 23]. This does not seem to influence the occurrence of an RVI of TALH cells as our results indicate. Therefore, a reduced transport capacity of potassium via the cell membrane does not seem to impair volume regulation and thus urea uptake. In addition, obstructing the $\text{Na}^+\text{-K}^+$ ATPase by ouabain circumvents reaccumulation of the potassium diffused from the cell, yet this potassium loss does not seem to influence the characteristics of the volume regulation within the measuring period.

Participation of an anion exchange system in the urea transport seems unlikely, because incubation with SITS displayed no distraction of the RVI.

The fact that the influence of the specific inhibitor of the $\text{Na}^+\text{-K}^+$ ATPase ouabain is lacking supports the idea that the RVI under hyperosmolar urea conditions is independent of the energy provided by ATP. Thus, a secondary active sodium-urea cotransport as described by Isozaki et al [2] for IMCD of rats with reduced protein diet does not seem to participate in urea transport in the thick ascending limb. In addition, this transport is phloretin-insensitive [2].

As observed with ouabain, inhibiting the $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ cotransport system in the apical membrane by furosemide

showed no visible influence on volume regulation of the TALH cells.

Inhibition of the $\text{Na}^+\text{/H}^+$ antiport and a sodium channel by amiloride did not change the cell volume compared to the control sample. An amiloride-inhibitable urea transport in the toad bladder has been reported [24, 25], but this may be due to an indirect effect caused by sodium transport inhibition [26]. Urea uptake is reduced under hyperosmolarity induced by NaCl in comparison to isoosmotic conditions. However, this decrease is not caused by the presence of a high NaCl concentration, but rather by the hyperosmolar conditions, as shown by the results of some experiments with hyperosmolarity induced by sucrose. Thus, it seems improbable that NaCl concentration or NaCl uptake influences urea transport in TALH cells. The fact that urea uptake remains unchanged under sodium-free conditions supports this premise.

These results indicate that neither Na, nor Cl, nor K are directly involved in the volume regulation. Furthermore, a link between urea transport and other known transport systems seems rather unlikely.

In red blood cells, thiourea is a relatively effective inhibitor of urea transport [27–29], while methylurea and acetamide are less effective and nearly equipotent, in contrast to rat [26] and rabbit [30] IMCD. However, we found no significant differences in the effectiveness of inhibition between these urea analogs in TALH-cells, but the results are consistent with an interaction of urea analogs and urea as a specific transport.

Antidiuretic hormone (ADH) causes an increase of adenylatcyclase activity in the thick ascending limb. Incubating the TALH cells with cAMP (2nd messenger for the effect of ADH in the range of the medullary section of the thick ascending limb) does not significantly change the RVI. Rocha and Kokko [11] could not prove an increase of urea permeability in the medullary ascending limb in the presence of ADH either. Thus, ADH does not seem to have any influence on urea uptake, as it is described for the inner medullary collecting duct of rats [31, 32], of the TALH.

Comparison of the described urea transport systems

The rat UT1 [1] shows the same functional characteristics as the vasopressin-sensitive urea transport of the IMCD described by Knepper and Star [33] as well as by Sands, Nonoguchi, and Knepper [32]. Chou et al found no saturation in the range between 0 and 800 mM, tested in rat's IMCD [34]. In this study, we examined up to urea concentrations of 600 mM without any signs of a saturation. However, the urea permeability found in IMCD [35] is considerably higher than the values found for the TALH cells. UT1 is almost completely inhibitable by phloretin and urea analogs, but is not affected by pCMBS [1]. In this study, we could not determine any effect of vasopressin simulated by a cAMP incubation, but the UT1 transport is

vasopressin-sensitive [1]. In rat, UT1 is exclusively expressed in the IMCD [5]. This and the missing vasopressin influence points against a responsibility of UT1 for urea transport in TALH cells. Furthermore, the urea permeability in the IMCD, which predominantly is due to UT1, shows a dependence on osmolarity. Raised osmolarity would lead to an increased urea permeability [34, 21], in contrast to our results.

You et al cloned and described [30] the rabbit UT2 transport. This UT2 transport displayed no saturation characteristics up to the maximum tested concentration of 200 mM urea [30]. Thiourea and N-methylurea inhibited UT2 approximately in the same range as the uptake of the TALH cells, but unlike UT2, the uptake can be reduced by acetamide. According to You et al, 0.35 mM phloretin inhibited the urea transport of UT2 by 48%, and 0.7 mM by 78% [30]. In accordance with these values, the urea uptake of TALH cells decreased 31% in the presence of 0.5 mM phloretin. UT2 showed no sensitivity to pCMBS [8]. Like the urea uptake in TALH cells, UT2 possesses no sodium or chloride dependency. No influence of cAMP to the transport in oocytes injected with UT2 was shown [30]. Shayakul et al showed that in rat UT2 is exclusively expressed in descending thin limbs in outer and inner medulla [5]. Therefore, it seems impossible that UT2 is responsible for urea transport in TALH cells, although the pattern of inhibition and the transport kinetic shows some similarities.

The human urea transport HUT11 is existent in several tissues and in red blood cells [36, 37]. Recently it was reported that HUT11 in rat and human kidney is expressed in the endothelial cells of the inner and outer medullary vasa recta exclusively [7], compatible with the urea transport in rat's vasa recta, described by Pallone [6]. HUT11 is inhibited by phloretin and pCMBS, furthermore by urea analog like thiourea [8] and it is not controlled by vasopressin [7, 36]. The rat homolog UT3 [8] shows similar functional characteristics and was localized in the descending vasa recta of the inner stripe of the outer medulla, the papillary surface epithelium and the ureter. There is a functional resemblance between the transport characteristics described in this study and the characteristics of HUT11 and UT3, but the localization points against a responsibility of UT3 for urea transport in TALH cells.

The human urea transport HUT2 is present in the inner medullary collecting duct [38]. Interestingly, it shows no signs of pCMBS sensitivity, as UT1, whereas phloretin leads to a significant inhibition of urea uptake. Olives et al suggested HUT2 to be vasopressin controlled, but no further functional test results are available yet [38].

Urea transport in TALH cells

The apical membrane of the TALH is almost water impermeable [11, 39]. Thus, specific water channels should not occur. It seems impossible that the urea transport

should work similar to the aquaporin-3 channel described by Ishibashi et al [3] at the collecting duct section, which, unlike other water channels, is not water selective but rather transports water and small non-electrolytes like urea. The strong tenfold increase of urea transport at 37°C indicates the presence of specific structures that allow urea uptake. Despite a small permeability coefficient, the rapid RVI and the rapid equilibration, as well as the high specific uptake, could be caused by the presence of a specific urea transport system. If those transports were lacking, urea exchange would be considerably more difficult due to the low urea diffusibility. We can only suspect that the low urea diffusibility is caused by the fact that water impermeability is necessary. The thesis of a transport is supported by the inhibition of urea uptake by phloretin and urea analog.

Physiological relevance

Due to the low permeability, it seems impossible to achieve a physiologically significant participation in the urea circulation within the nephron, as it is described for the cortical TALH [9]. But the high and variable osmolarities that are predominant in the lumen of the tubule require a rapid volume regulation of the TALH cells to changing osmolarity conditions. This effect is achieved by a fast equilibration of extracellular urea gradients via the cell membrane and by a rapid urea uptake.

However, the medullary TALH plays an important role in urea recycling by avoiding great urea losses out of the lumen. The medullary TALH contributes to the maintenance of a high urea concentration in the inner medulla, because high urea concentrations are delivered to the cortical segment. Urea partly might enter the descending limb, or may be conveyed to the inner medulla via the inner medullary collecting duct.

ACKNOWLEDGMENTS

This work is part of the M.D. thesis of C.H. Reisse, and was supported by a grant from the Deutsche Forschungsgemeinschaft #Gr 916. We thank Prof. Dr. R.K.H. Kinne for helpful discussion. The technical assistance of Mrs. Elke Brunst-Knoblich is gratefully acknowledged.

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