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Urea Inhibits the Na-K Pump in Human Erythrocytes

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Abstract. Recent studies have established that urea alters the activity of several volume-sensitive cation transport pathways. However, it has remained unclear whether urea has any effect on transport pathways that are not volume-sensitive. We examined the effect of urea on Na-K pump in the human erythrocytes. In cells from nine subjects, 500 mM urea inhibited 52 ± 10% of the pump activity measured as the ouabain-sensitive (OS) K influx. Urea inhibited the OS K influx reversibly, in a concentration-dependent manner. [^{3}H] oubain binding, a measure of the number of Na-K pump sites remained unchanged with urea. Urea decreased the $V_{\max}$ for ouabain-sensitive K influx, but did not alter the apparent K_m for external K. Furthermore, urea did not alter the apparent K_m for intracellular Na. The ion turnover per pump site was decreased in the presence of urea. Thus, physiologically relevant urea concentration inhibit the Na-K pump in human erythrocyte. The inhibition of the Na-K pump by urea suggests that the effects of urea may not be limited to volume-sensitive transporters, but may be more widespread.

Key words: Na-K-ATPase

Introduction

Recent studies have shown that subdenaturing concentrations of urea (<1 M) affect a variety of volume-sensitive transport pathways. Thus, urea has been shown to activate KCl cotransport in dog, sheep and human erythrocytes (Dunham, 1995; Kaji & Gasson, 1995; Parker, 1993), inhibitts Na/H exchange in the dog erythrocytes (Parker, 1993) and the NaK2Cl cotransport in human erythrocytes (Kaji & Gasson, 1995) and in mouse medullary thick ascending limb cells (Kaji, Diaz & Parker, 1997). The mechanism by which urea affects volume-sensitive transporters has not been established with certainty. However, recent evidence supports the view that urea, perhaps by its effect on macromolecular crowding, inhibits kinase activity and causes a net dephosphorylation of the affected volume-sensitive transporters, or of some key regulatory enzyme (Dunham, 1995; Kaji & Gasson, 1995).

While the effect of urea on volume-sensitive transporters is now well established, it has remained unclear whether urea inhibits transport pathways that are not volume-sensitive. The Na-K pump is not generally considered to be volume-sensitive, but its activity in renal tubular epithelial cells is modulated by kinases and phosphatases (Bertorello et al., 1991; Satoh, Cohen & Katz, 1993). The finding that urea alters the activity of certain kinases (Cohen & Gullans, 1993; Meister et al., 1989), coupled with the finding that the Na-K pump activity is modulated by phosphorylation, raised the possibility that urea may alter the activity of the Na-K pump. Therefore, in this study, we examined the effect of urea on Na-K pump activity in human erythrocytes.

Materials and Methods

Venous blood from nine normal donors was used fresh or within 24 hr of collection. Cells were separated from plasma and washed 3 times with washing solution A (NaCl 145 mM, KCl 5 mM, dextrose 5 mM, Hepes 5 mM, pH 7.4 at 37.4°C) or with other media specified in table or figure legends. Relative cell volume (RCV) was measured from the change in mean cell hemoglobin concentration (MCHC) as described previously (Cheng, Kahn & Kaji, 1984; Kaji, 1993; Kaji & Gasson, 1995; Lim, Gasson & Kaji, 1995).

Intracellular Na, K and Water Content

Cell electrolytes were measured by flame photometry of lysates from washed cells as described in previous publications (Cheng et al., 1984;

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Kaji,1993; Kaji & Gasson, 1995; Lim et al., 1995). Water content of fresh cells was measured by drying 0.1 ml of wet cells to a constant weight in an oven at 100°C for 24 hr (Cheng et al., 1984; Kaji, 1993; Kaji & Gasson, 1995; Lim et al., 1995). Under conditions of rapid change in cell volume, the relative cell volumes were calculated from their mean corpuscular hemoglobin concentration (MCHC) as described (Cheng et al., 1984).

Alteration of Cell Electrolytes

The nystatin method of Cass and Dalmark (1973) was used to vary the ionic composition. Cells were incubated at 4% hematocrit in a medium containing 150 mM (KCl + NaCl), 33 mM sucrose, 20 mg/liter nystatin, 2.5 mM HEPES, pH 7.2 at 4°C for 15 min in the dark. The Na concentration in the medium was varied reciprocally with K to obtain the desired intracellular Na concentration. The procedure for washing cells and eluting nystatin has been described in detail previously (Cheng et al., 1984; Kaji, 1993; Lim et al., 1995). After washing, cell aliquots were stored overnight at 4°C in media containing (in mM) NaCl 10, KCl 140, glucose 5, Hepes 5, pH 7.4. The next morning, cells were washed three times with 150 mM N-methyl glucamine Cl, pH 7.4 and then divided in two aliquots. One aliquot was used for measurement of Na, K, water content and MCHC, whereas the other aliquot was used for K influx.

K Influx

K influx was measured as described previously (Cheng et al., 1984). Washed cells (2–4% hematocrit) were suspended in a medium containing (in mM) NaCl+KCl 150, dextrose 5, Tris.HCl 5, pH 7.4, with or without 0.1 mM ouabain. ^{86}Rb (0.1 μCi/ml) was added at zero time and the transport was continued in the absence or presence of 10 μM bumetanide. Unless specified otherwise, studies in the presence of urea were performed by pretreating cells for 1 hr prior to the addition of ^{86}Rb, and urea was also included in the flux medium. K uptake was terminated at 60 min (6 min for experiments described in Fig. 4) by washing the cells four times with ice-cold 0.115 M $MgCl_2$. Cell pellets were lysed with ice-cold Triton X-100 (0.1% vol./vol.), followed by protein precipitation with trichloracetic acid (5% wt./vol.), and centrifugation for 10 min at 3,000 × *g*. The radioactivity of the supernatant solution was counted by Cerenkov radiation in a scintillation counter as previously described by Dunham and Ellory (1980). The Na-K pump activity was defined as the ouabain-sensitive (OS) K influx, and was defined as the difference in K influx with and without 0.1 mM ouabain. K influx rates were expressed as mmol per liter original cell per hour ($mmol \cdot loc^{-1} \cdot h^{-1}$).

[^{3}H] Ouabain Binding

The techniques for ascertaining the specific activity of [^{3}H] ouabain, for measuring the nonspecific binding of ouabain, and for ascertaining that the radioactivity was attached to the ouabain molecule have been described previously (Kaji, 1981). [^{3}H] ouabain binding was measured by the method previously described (Joiner & Lauf, 1975), with minor modifications as previously described (Cheng et al., 1984). Cells were incubated for 3 hr with 100 nM [^{3}H] ouabain binding (specific activity 12–18 Ci/mmol) in a K-free medium containing NaCl 150 mM, $MgCl_2$ 1 mM, dextrose 5 mM, Tris.HCl 5 mM, pH 7.4 at 37°C. After incubation, cells were separated from unbound [^{3}H] ouabain by density gradient, lysed, and washed twice to remove hemoglobin. The cell membranes were solubilized in 0.1 N NaOH, treated with HCl to reduce chemiluminescence, and the β-radioactivity was counted in 10 ml NEN-989 in a scintillation counter. [^{3}H] ouabain binding sites per cell were calculated as described previously (Kaji, 1981). Nonspecific [^{3}H] ouabain binding was measured as the [^{3}H] ouabain binding in the presence of excess (0.1 mM) unlabeled ouabain, and substracted from total ouabain binding to derive specific ouabain binding.

Materials

^{86}Rb (as the chloride salt) and NEN-989 were purchased from Dupont-NEN (Boston, MA). All other reagents were purchased from Sigma Chemical, St. Louis, MO, or J.T. Baker through VWR Scientific, Piscataway, NJ. Urea was dissolved in media on the day of the experiment. Urea-containing media were never stored for >48 hr. When reagents were dissolved in dimethyl sulfoxide (DMSO), the amount of DMSO added to flux media never exceeded 0.5% (vol./vol.).

Statistical and Kinetic Analysis

Experimental datapoints were fitted to equations using linear or nonlinear regression with successive iteration using Sigmaplot (Jandel Scientific, Cortes Madera, CA). The kinetic parameters V_{max} and K_m for active pump mediated K influx were calculated using a modified form of the Hill equation: $v = V_{max}/(1 + [K_m/S])^n$, where v is the rate of active transport at a given ion concentration S, V_{max} is the maximal pump rate, K_m is the ion concentration required for half maximal active pump rate, and n is the Hill coefficient. This equation has been used by previous investigators, who showed that with certain assumptions, n was equal to the number of binding sites (Garay & Garrahan, 1973). The Hill coefficient was taken to be 2 for K and 3 for Na. Results are presented as mean ± SD or mean ± SE as specified in Results of figure legends. The standard deviation (SD) or ouabain-sensitive K influx was calculated as $\sqrt{(SD1)^2+(SD2)^2}$ where (SD1) and (SD2) represent SDs of total and ouabain-resistant influx respectively. When the standard error of the calculated kinetic parameters (V_{max} or apparent K_m) was >12%, the data were rejected.

Results

Urea equilibration across erythrocyte membranes is mediated by a facilitated diffusion process and is extremely rapid (Macey, 1984). Therefore, the addition of urea (100–600 mM) would not be expected to alter RCV. We confirmed that RCV was unchanged 2 min after addition of 600 mM urea to erythrocyte suspensions (0.98 ± 0.02 *vs.* 0.99 ± 0.02, $n = 4$). Similarly, RCV was unchanged after 120 min of incubation with urea.

We measured ouabain-sensitive (OS) K influx in cells from nine normal subjects in the absence and presence of 500 mM urea. Each data point is a mean of three separate determinations. In the absence of urea, OS K influx in erythrocytes was 1.01 ± 0.14 $mmol \cdot loc^{-1} \cdot h^{-1}$ (left, Fig. 1). In the presence of 500 mM urea, OS K influx was inhibited from 34%–75% in different individuals. The average inhibition was 52 ± 10% (Fig. 1). The variation in the percent inhibition was largely due to inter-individual variations; the percent inhibition in the same subject on different days varied by 7% or less (*not shown*).

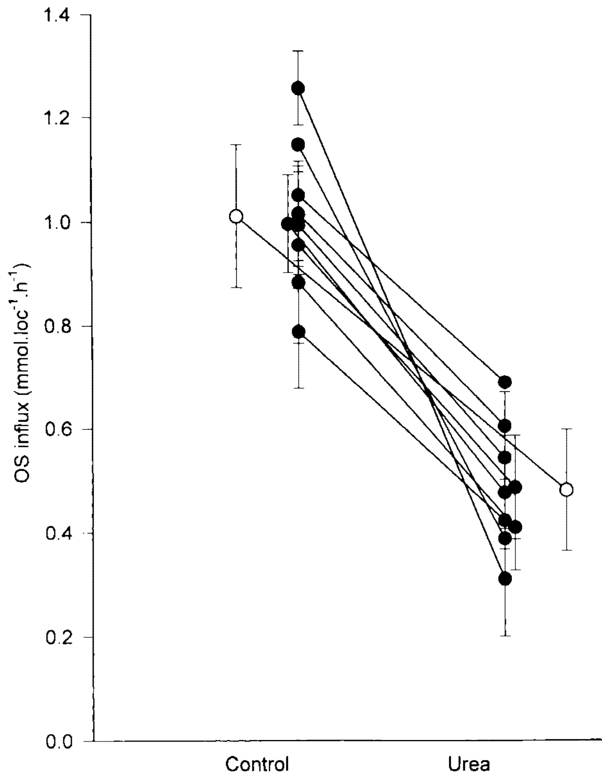

Fig. 1. Effect of 500 mM urea concentration on OS K influx in nine different normal subjects. The hollow circles depict the mean for the control and urea groups. Each filled cicle depicts the average ± SD of triplicates from a single individual obtained on a given day. Some filled circles are offset slightly to the left (control) or right (urea) to avoid overlap.

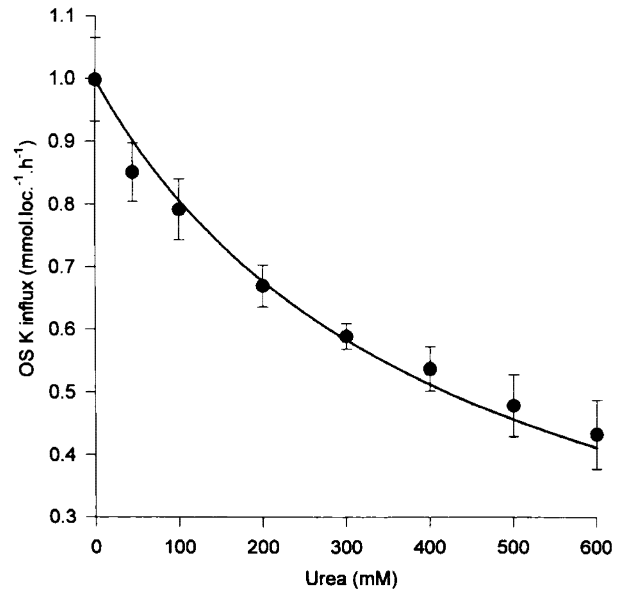

Fig. 2. The effect of various urea concentrations on OS K influx in human erythrocytes. Cells were exposed to various urea concentrations for 60 min and OS sensitive K influx was measured as described in Materials and Methods. Depicted are means ± SE from 3 separate experiments from a single individual. Similar results were obtained in two other subjects. The solid line represents the theoretical line derived from fitting the data points to the equation, $Ju = Jc \cdot Ki/(Ki + U)$, where Ju and Jc represent OS K influx in the presence and absence of urea, Ki represents the urea concentration required to inhibit 50% of the bumetanide-sensitive K influx, and U represents the urea concentration in mM.

The concentration-dependence of urea inhibition was determined in three subjects. Figure 2 shows the results in one subject. At a concentration of 50 mM, which is frequently observed clinically in uremic subjects, the inhibition was 15%. About 60% of the OS K influx was inhibited at a urea concentration of 600 mM. The concentration required for 50% inhibition of OS K influx was 435 ± 18 mM .

To further define the effects of relatively low urea concentrations, we performed K influx assays in cells from 6 different subjects in the absence and presence of urea. Cells from each subject were divided into 2 aliquots, and K influx was performed simultaneously in the absence and presence of 45 mM urea, a concentration commonly observed in uremic subjects clinically. We observed a small (11 ± 2%), but significant inhibition of OS K influx with 45 mM urea (0.89 ± 0.09 *vs.* 1.02 ± $0 \cdot 11$ mmol $\cdot$ loc$^{-1} \cdot$ h^{-1}, $P < 0.0002$).

Next, we performed experiments to assess whether the urea-mediated pump inhibition was reversible. Figure 3 shows the OS K uptake in control cells (cell not exposed to urea), cells left in 500 mM urea, and cells exposed to 500 mM urea and then washed free of urea (Fig. 3). The reversibility of urea-induced pump inhibition rules out irreversible cell damage as a cause of the observed inhibition, and is consistent with the notion that urea may act at the phosphorylation-dephosphorylation step to exert the inhibition of the pump.

Phosphorylation-dephosphorylation events change transport after a time delay, and analysis of the delay has provided useful insights into the regulation of transport (Dunham, 1995; Jennings & Al-Rohil, 1990; Kaji & Gasson, 1995). We examined the time course of inhibition of the pump after addition of urea (Fig. 4). About 20% of the OS K influx was inhibited at the earliest time point (20 min), and maximum inhibition was observed at ~60 min after initial exposure to urea. The long delay before the maximum inhibition is consistent with an action at the phsophorylation-dephosphorylation step, and suggests that a direct inhibitory effect of urea on the Na-K-ATPase is unlikely.

To examine whether urea inhibted the pump activity by altering substrate affinity or by decreasing V_{max}, we examined the dependence of OS K influx on external K and intracellular Na in the presence and absence of 500 mM urea. Figure 5 shows the dependence of OS K influx on external K in the absence (hollow circles) and pres-

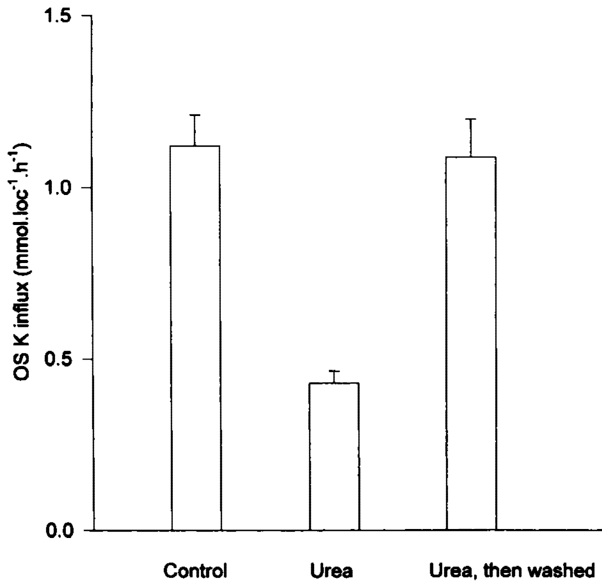

Fig. 3. Reversibility of urea effect. Cells were not exposed to urea (control, bar on left), preincubated with 500 mM urea, which was also present in the flux assay medium (middle bar), or exposed to 500 mM urea and then washed three times with urea-fee medium for 15 min (total) before the addition of ^{86}Rb (right bar). Mean ± SD of two experiments on a single donor.

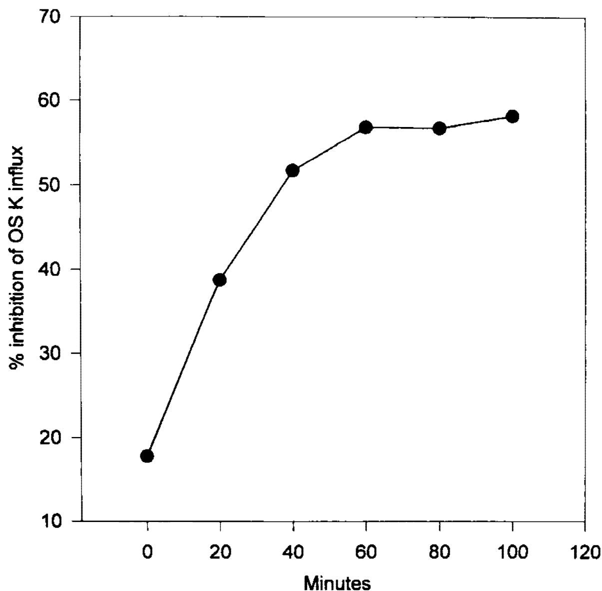

Fig. 4. Percent inhibition of OS K influx as a function of time of exposure to 500 mM urea. The flux assay was terminated at 6 min for this experiment. Each point is a mean of triplicates on the same day from a single individual.

ence (filled circles) of 0.5 M urea. In the absence of urea, the V_{max} was 0.78 ± 0.06 mmol · loc^{-1} · hr^{-1} and the apparent K_m was 1.10 ± 0.02 mM. In the presence of urea, the V_{max} was lower (0.37 ± 0.04 *vs.* 0.78 ± 0.06),

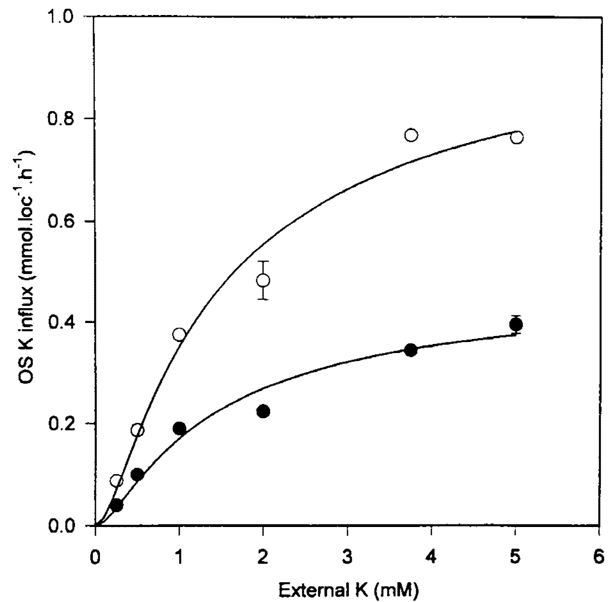

Fig. 5. Dependence of OS K influx on extracellular K concentration. Hollow circles represent datapoints from cells not exposed to urea. Filled circles represent data from cells exposed to urea for 60 min before the addition of ^{86}Rb, and during the flux assay. The solid lines represent the theoretical line derived from a nonlinear regression equation using a two-site equation $v = V_{max}/(1 + (K'_K/K_o)^2)$ were v is the rate of OS K influx, V_{max} is the maximal pump rate, K_o is the external K concentration, and K'_K is the dissociation constant for K_o. Error bars were omitted when smaller than symbols.

but the apparent K_m for external K was not altered (1.06 ± 0.19 *vs.* 1.10 ± 0.02 mM).

To examine the dependence of pump activity on intracellular Na, we used the ionophore nystatin, which does not alter the properties of the erythrocytes with respect to the OS K influx (Cass & Dalmark, 1973). Intracellular Na was varied reciprocally with K. We confirmed that in our hands, erythrocyte volume was unchanged after nystatin method (RCV was 1.02 ± 0.03 in nystatin treated cells). In the absence of urea (hollow circles, Fig. 6), the V_{max} was 2.04 ± 0.19 mmol · loc^{-1} · hr^{-1} and the apparent K_m was 9.0 ± 1.5 mM. In the presence of urea (filled circles, Fig. 6), the V_{max} was lower (0.94 ± 0.09 *vs.* 2.04 ± 0.19 mmol · loc^{-1} · hr^{-1}), but the apparent Km for intracellular Na was not altered (8.9 ± 1.1 *vs.* 9.0 ± 1.5 mM).

To test the possibility that urea decreased the number of Na-K pump sites per cell, we measured maximum [^{3}H] ouabain binding in the presence and absence of 0.5 M urea. The total, nonspecific and specific [^{3}H] ouabain binding were unchanged in the presence of urea (Table 1). The ion turnover per site was calculated from the V_{max} obtained from Fig. 6 at saturating intracellular Na and extracellular K concentrations, and the maximum saturable Na-K pump sites in cells from the same donor. The K ion turnover per site per minute was 4482 in the

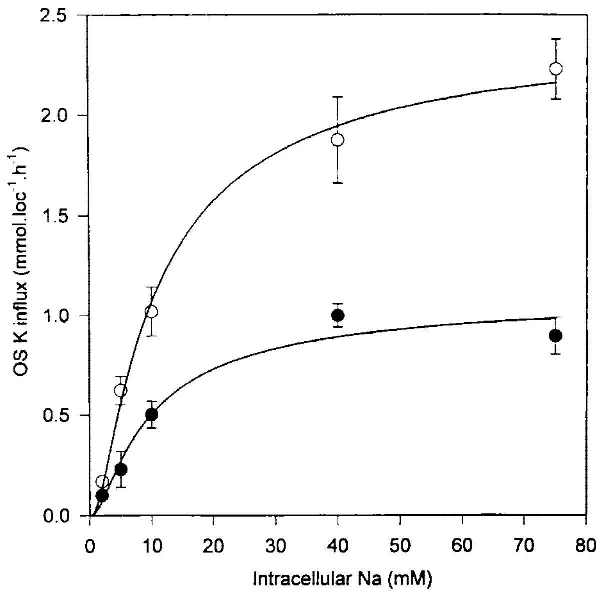

Fig. 6. Dependence of OS K influx on intracellular Na concentration. Hollow circles represent datapoints from cells not exposed to urea. Filled circles represent data from cells exposed to urea for 60 min before the addition of ^{86}Rb, and during the flux assay. The solid lines represent the theoretical line derived from a nonlinear regression equation using a three-site equation $v = V_{max}/(1 + (K'_{Na}/Na_i)^3)$ where v is the rate of OS K influx, V_{max} is the maximal pump rate, Na_i is the intracellular Na concentration and K'_{Na} is the dissociation constant for Na_i.

Table 1. Effect of urea on Na-K pump sites

	Control	Urea (500 mM)
[^{3}H] ouabain binding, total	406 ± 15 (4)	393 ± 20 (40)
[^{3}H] ouabain binding, nonspecific	10 ± 2 (4)	8 ± 1 (4)
[^{3}H] ouabain binding, specific	396 ± 15 (4)	385 ± 20 (4)
V_{max} (from Fig. 6)	2.04 ± 0.19	0.94 ± 0.06
K ion turnover per pump site per minute	4482	2120

[^{3}H] ouabain binding was expressed as sites per cell, and V_{max} as mmol · loc^{-1} · hr^{-1}. Using the Avogadro number and mean corpuscular volume, V_{max} was recalculated as ion turnover per cell per minute. This number was divided by the number of Na-K pump sites per cell to derive the K ion turnover per pump site per minute.

absence of urea, in agreement with results reported earlier (Cheng et al., 1984). Urea reduced K ion turnover by ~53% (2120 *vs.* 4482 ions per site per min, Table 1).

To ascertain whether phosphorylation regulates the pump activity in human erythrocytes, we examined the effect of okadaic acid, an inhibitor of protein phosphatase 1 and 2A. At a concentration of 50 nM, which is near the K_i for inhibition of K-Cl cotransport in human erythrocytes, okadaic acid inhibited 55% of the OS K influx (Jennings & Schulz, 1991; Kaji & Tsukitani, 1991). At a concentration of 400 nM, which is the maximum effective concentration for its action on K-Cl cotransport, the pump activity was completely abolished (Table 2). The effects of submaximal concentrations of okadaic acid (50 nM) and urea (200 mM) were additive (Table 2).

Table 2. Effect of phosphatase inhibitors and urea on Na-K pump

	OS K influx (mmol · loc^{-1} · hr^{-1})
Control	1.03 ± 0.06
Vehicle alone (DMSO 0.05%)	0.99 ± 0.09
Okadaic acid (50 nM)	0.45 ± 0.04
Okadaic acid (400 nM)	0.04 ± 0.04
Okadaic acid (50 nM) + Urea (200 mM)	0.10 ± 0.02
Okadaic acid (50 nM) + Urea (500 mM)	0.06 ± 0.05

Okadaic acid and urea were added 1 hr before the addition of ^{86}Rb. Okadaic acid and calyculin were dissolved in 0.05% DMSO. The results are mean ± SD for 4 datapoints on a single individual.

Discussion

Urea inhibited the Na-K pump in human erythrocytes reversibly, and in a concentration-dependent fashion. Recent evidence suggests that the regulation of the Na-K pump is dependent on phosphorylation-dephosphorylation state of the Na-K pump complex. In general, phosphorylation inactivates the pump, whereas dephosphorylation stimulates the pump. Thus, in proximal tubular cells, dopamine-activation of cAMP-dependent protein kinase was shown to result, sequentially, in phosphorylation of DARPP-32, inhibition of protein phosphatase (PP)1, a net phosphorylation of the Na-K pump (or a closely associated regulatory protein), and a decrease in pump activity (Meister et al., 1989). In medullary thick ascending limb and cortical collecting duct cells, inhibition of the protein phosphatase (PP) activity (of calcineurin) by FK-506 led to the inhibition of Na-K-ATPase activity (Lea et al., 1994). We found that okadaic acid (400 nM), an inhibitor of PP1 and PP2A completely inhibited OS K influx in human erythrocytes (Table 2). Thus, the Na-K pump in the erythrocyte is also inhibited by phosphorylation. Thus, if urea-mediated inhibition of the pump was caused by a net phosphorylation of the Na-K pump complex, urea must have stimulated a kinase or inhibited a phosphatase.

It is unclear whether urea-mediated pump inhibition is related to stimulation of a regulatory kinase in the erythrocyte. There is precedence for urea stimulation of a kinase in nucleated cells. In inner medullary collecting duct cells, urea has been shown to stimulate transcription of Egr, a zinc finger nuclear protein, by activating a

variety of protein kinases (Cohen & Gullans, 1993). Urea activates protein kinase C and ribosomal S6 kinase (RSK) in the mouse inner medullary collecting duct cells by separate transcriptional mechanisms (Zhang & Cohen, 1998). However, this effect is cell-specific; non-renal epithelial cells and non-epithelial renal cells did not show this effect (Cohen & Gullans, 1993).

Could the observed pump inhibition be secondary to inhibition of a phosphatase? It is known that urea competitively inhibits a variety of enzymes, both by macromolecular-solvent interactions (Yancey et al., 1982), and directly, in dilute solutions, by a mechanism unrelated to macromolecular crowding (Rajagopalan, Fridovich & Handler, 1993). There are also hints in the literature that urea may inhibit protein phosphatase in the human erythrocyte. In previous studies on the urea-mediated activation of K-Cl cotransport in human erythrocytes, it was observed that as urea concentrations were raised from 400 to 600 mM, the time lag of activation increased 10–20 fold, but the flux rate increased only modestly, about 2-fold (Kaji & Gasson, 1995). We postulated, that at high concentrations, urea may have inhibited the phosphatase as well as the kinase, so that the activating effect of kinase inhibition may have been blunted by the inhibition of phosphatase. Therefore, the possibility that inhibition of a phosphatase accounts for the observed pump inhibition must be considered.

Urea-mediated modulation of other volume-sensitive transporters has been attributed to urea-protein interactions. While macromolecular crowding increases the activity coefficient of proteins, (i.e., increases the chemical potential of proteins, including enzymes, at fixed concentrations), urea has the opposite effect. It decreases the chemical potential of proteins at fixed concentrations (Kaji et al., 1997; Lim et al., 1995; Parker, 1993; Parker, Dunham & Minton, 1995). It has been proposed that urea affects the activity of the volume-sensitive transporters by decreasing the chemical potential of a key regulatory kinase. This, in turn leads to a diminished tendency of the kinase to associate with, and phosphorylate, the membrane ion transporter. Because the phosphatase is tightly attached to the membrane, its chemical potential is presumably unaffected (Bize et al., 1998; Kaji et al., 1997; Parker, Dunham & Minton, 1995). The resulting net dephosphorylation alters the activity of the volume-sensitive transporter. It is unlikely that urea inhibited the Na-K pump by such a mechanism, because pump inhibition implies a net phosphorylation of the pump complex, caused either by kinase stimulation, or phosphatase inhibition, and neither can be readily explained by the compensation for macromolecular crowding by urea.

Urea has been known to inhibit a variety of enzymes directly, in dilute solutions, independent of macromolecular-solute interactions (Rajagopalan et al., 1993). The observed results could be explained by postulating a direct competitive inhibition of Na-K-ATPase activity by urea. However, direct inhibition of the Na-K-ATPase activity is very unlikely in light of the long (90 min) lag time for urea to inhibit the pump.

It is long known that the Na-K pump is inhibited in erythrocytes from uremic subjects (Kaji & Kahn, 1987). Based on the strong inverse correlation between the increase in cell Na and the decrease in the number of pump sites, we suggested that a decrease in the synthesis of Na-K pump sites was the major defect in uremia (Cheng et al., 1984; Kaji & Kahn, 1987). However, other investigators also noted a direct, acute inhibition of the pump activity upon incubation with uremic predialysis (but not postdialysis) plasma (Izumo et al., 1984; Kelly et al., 1986; Quarello et al., 1985; Zannad et al., 1982). Based on these findings, it was postulated that a dialyzable toxin may directly inhibit the Na-K pump. In this study, at blood urea levels commonly encountered in uremic subjects (~45 mM), a small (11%) but statistically significant pump inhibition was observed. Thus, a portion of the inhibition observed with uremic plasma may be attributable to the high urea concentration in the uremic plasma. It is conceivable that over an extended period of time, even a small inhibition of pump may result in adverse effects. Assuming that the effect seen in erythrocytes is representative of a similar inhibition of the pump in other tissues, such as the skeletal muscle, increased blood urea concentrations may have widespread deleterious effects. The finding of pump inhibition, taken together with the inhibition of NaK2Cl cotransport and the stimulation of KCl cotransport, with urea concentrations normally prevalent in the uremic plasma, buttresses our suggestion that urea is not only a marker for accumulation of other uremic toxins, but may be a significant uremic toxin itself (Kaji et al., 1997; Kaji & Gasson, 1995; Lim et al., 1995).

Urea is the end product of protein catabolism in mammals, and makes up ~50% of the urinary solutes in human subjects consuming a normal protein diet (about 60–80 g/day). Because of recycling of urea in the renal medulla, urea concentration, normally about 3–4 mM in blood and extracellular fluids, increases along the corticomedullary axis of the mammalian kidney. Urea concentrations of 400–900 mM have been measured at the tip of the dog renal papilla (Ullrich & Jarausch, 1956), and similar findings have now been obtained in a variety of mammalian species, including rats, rabbits, hamsters and sheep (Knepper & Rector, 1996). The effects of urea on the Na-K pump are physiologically relevant because the erythrocytes are exposed to high urea concentrations (up to 600 mM in man) during their passage through the vasa recta in the renal medulla. The effects of repeated circulation of erythrocytes through an environment with high ambient urea concentrations need further studies.

In addition, the effects of high medullary urea concentrations on the Na-K pump activity of the renal tubular epithelial cells of the medulla have not been investigated. The findings presented here emphasize the need for a detailed examination of the effects of high urea concentrations, and of sudden changes in urea concentrations, on the Na-K pump activity of the tubular epithelial cells of the renal medulla.

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