STEADY STATE WATER AND SOLUTE REGULATION IN PAPILLARY COLLECTING TUBULE CELLS AND INTERSTITIAL CELLS OF *Rattus norvegicus*.

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We have been able to isolate and culture papillary collecting tubule cells (RPCT) and interstitial cells (RPIC) from Munich Wistar rats. We have also begun to measure steady state water and solute maintenance in RPCT and RPIC as a function of medium composition.

RPCT were isolated using a modification of the method of Sato and Dunn, Am. J. Physiol. 247:F423-F433, 1984; RPIC were isolated using the method of Dunn et al., Prostaglandins 12:37-49, 1976. Primary cultures of both cell types were grown in 60 mm culture dishes at 37° C and 95% air - 5% CO_2. The basic culture medium used was Dulbecco's Modified Eagle Medium (pH 7.4) supplemented with 25 mM HEPES, 42 mM $NaHCO_3$, 10% FBS, 2 mM glutamine, and 1 ml/l Antibiotic-Antimycotic Solution. For RPCT, Na and urea were added to final concentrations of 280 mM and 330 mM, respectively (= High Na - High Urea Medium); for RPIC, urea was added to a final concentration of 30 mM (= Control Medium). The concentrations of Na and urea in media reflect the concentrations of these solutes found in papillary tissues (Schmidt-Nielsen et al., Am. J. Physiol. 244:F472-F482, 1983). Seven day sub-confluent primary cultures were then gradually acclimated to either the Control or the High Na - High Urea Medium. Cells were harvested after they had been in a final medium for 24 hours (day 10). We examined RPCT and RPIC, each acclimated to the 2 media, simultaneously in a given experiment (n = 4 to 6). We obtained the same results in duplicate experiments which we performed separately.

Cells were harvested in 0.05% trypsin, washed and suspended in Ringer's solutions (pH 7.4) containing 1 mM polyethylene glycol (MW 4000, PEG) and having the same inorganic ion and urea concentrations (mM/l) as the media to which cells were acclimated (Control = 345 mOs/kg H_2O, 155 Na, 5 K, 159 Cl, 30 urea mM; High Na - High Urea = 875 mOs, 280 Na, 5 K, 285 Cl, 330 urea). The final Ringer's solution additionally contained 3.0 µCi ^{14}C-PEG/ml as an extracellular space marker. Aliquots of the cell suspensions (approximately 4 mg of cells in 0.5 ml) were placed in 1.5 ml centrifuge tubes and incubated for 60 min in a Dubnoff shaker at 37° C. Suspensions were then centrifuged and an aliquot of the supernatant removed for analysis. The rest of the visible supernatant was aspirated off and the cell pellet weighed (to 1.0 µg) in a predried, preweighed aluminum foil envelope (stored over desiccant). The envelope containing cells was immediately placed in another desiccator lying in a 60° C oven. Cells were dried to constant weight and analyzed by the reconstitution method (Ferraris and Schmidt-Nielsen, J. Exp. Zool. 222:113-128, 1982; Schmidt-Nielsen et al., 1983). Cells and media were analyzed for osmolality, Na, K, and urea concentrations. Values obtained were corrected for dilution and extracellular space. Total cell water content (g H_2O/g solute free dry wt = g sfdw), corrected for extracellular space, was calculated after the method of Ferraris and Schmidt-Nielsen, 1982. The concentration of unidentified solutes (mOs/kg H_2O) was calculated after the method of Schmidt-Nielsen et al., 1983. Solute contents (µM or µOs/g sfdw) were calculated by multiplying the solute concentration in cell water (mM or mOs) by the total cell water content in g/g sfdw. Data are expressed as concentrations (mM/l) or content (µM or µOs/g sfdw). Based on trypan blue exclusion (assessed at the end of each experiment), more than 97% of the cells under each experimental condition were viable.

Water and Solute Maintenance

When we acclimated cells to either the Control or the High Na - High Urea Medium, the steady state water content (g H_2O/g sfdw) of RPIC was lower than that of the RPCT (Figs 1a, 1b). Like most cells, the RPIC and RPCT hyporegulated intracellular Na and hyperregulated K (mM/l). However, the RPIC and RPCT were strikingly different in the intracellular concentrations of the solutes we measured. RPIC contained a lower concentration of Na than RPCT but a higher concentration of K. The RPIC appeared to be a typical low Na - high K cell. In contrast, in RPCT, Na and K concentrations were nearly equal. These differences existed in spite of the fact that the sum of intracellular [Na] + [K] was the same in both cells. Intracellular [Na] + [K] was somewhat higher than that in the Control Medium, but lower than that in the High Na - High Urea Medium. When we compared the RPIC and RPCT in the High Na - High Urea Medium with those in the Control Medium, neither cell type demonstrated a significant change in Na or K concentration. These results indicate that the RPIC and RPCT regulate steady state inorganic ion concentrations very differently. Data suggest a greater Na "leak" or a lower Na,K ATPase activity in the RPCT.

We found another major difference between the RPCT and RPIC. When acclimated to either medium, the RPCT hyporegulated intracellular urea concentration (mM/l) (Figs. 1a, 1b). In contrast, the RPIC contained the same concentrations of urea as did the media. The urea gradient across the RPCT cell membrane in the Control Medium was 17.0 ± 0.7 (mM/l); that across the cell membrane in the High Na - High Urea Medium was 121.6 ± 14.3. The increase in the gradient was significant ($P < 0.001$). These data suggest that the RPCT transport urea out of the cell.

Water and Solute Regulation

In both the RPIC and RPCT, the observed changes in water content did not correspond with predictions based on the van't Hoff relation (Table 1; Mean ± S.E.) where, if no volume regulation takes place, the ratio between cell osmolality (Π) in the Control Medium and cell osmolality in the High Na - High Urea Medium should equal the ratio between cell water content (V) in the High Na - High Urea Medium and cell water content in the Control Medium ($\Pi_1/\Pi_2 = V_2/V_1$). However, in both the RPCT and RPIC, V_2/V_1 was significantly greater ($P < 0.01$) than Π_1/Π_2. Neither cell type appeared to regulate volume completely (V_2/V_1 would equal unity); the RPCT, however, appeared to regulate water content to a greater degree than the RPIC.

Table 1.	Π_1/Π_2 (medium)	Π_1/Π_2 (cells)	V_2/V_1 (cells)
RPIC	0.40 ± 0.003	0.44 ± 0.03	0.65 ± 0.04
RPCT	0.39 ± 0.003	0.43 ± 0.04	0.84 ± 0.09

As a validation of our method for determining intracellular osmolality it can be seen that Π_1/Π_2, as calculated for the measured osmolality of the cells, did not differ significantly from Π_1/Π_2, as calculated for the measured osmolality of the medium samples in which we incubated the cells (Table 1). The measured osmolalities, for example, in the RPCT cells and corresponding media were: 388 ± 41 (cells) versus 341 ± 2.5 (Control Medium) and 911 ± 38 (cells) versus 877 ± 1.2 (High Na - High Urea Medium) (mOs/kg H_2O; Mean ± S.E.).

In both the RPIC and RPCT, K content (μM/g sfdw) decreased with decreased water content (Figs. 2a, 2b). In the RPIC, the K content of the cells in

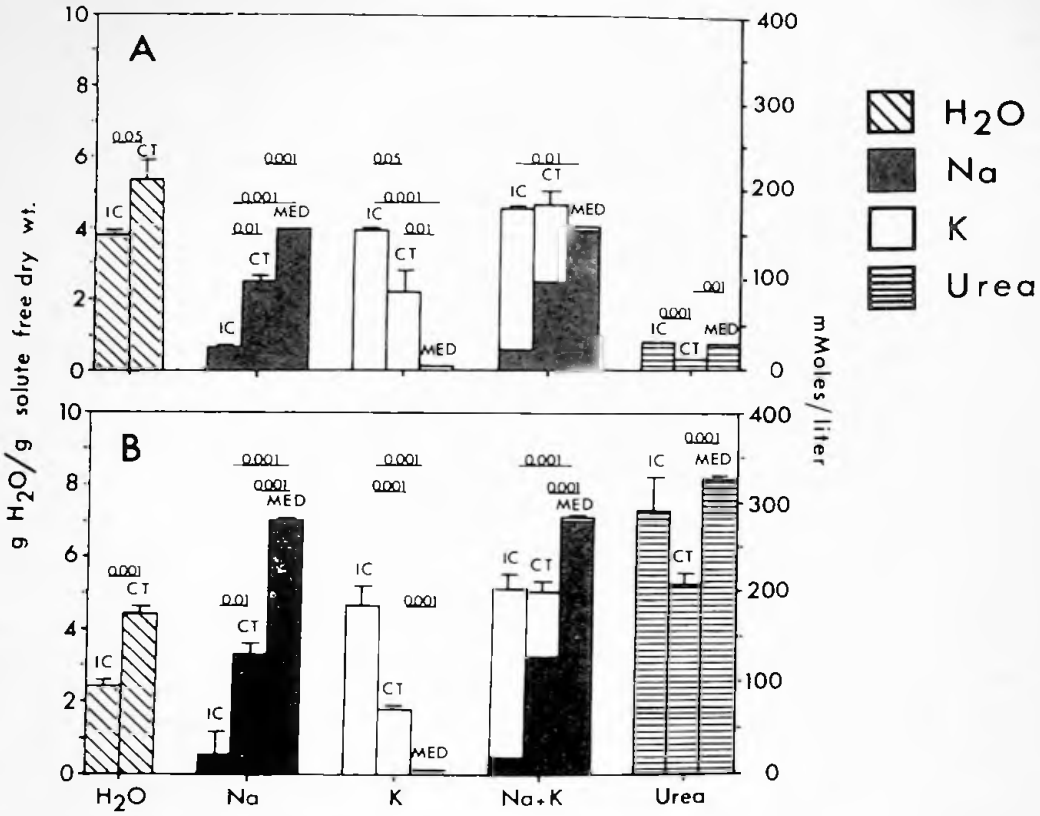

Figure 1. Steady state water content and solute concentrations (Mean + S.E.) in RPIC (IC) and RPCT (CT) acclimated to the Control Medium (A) or the High Na - High Urea Medium (B) (MED). Significance bars begin and end at values significantly different from each other ($P < 0.05 - 0.001$).

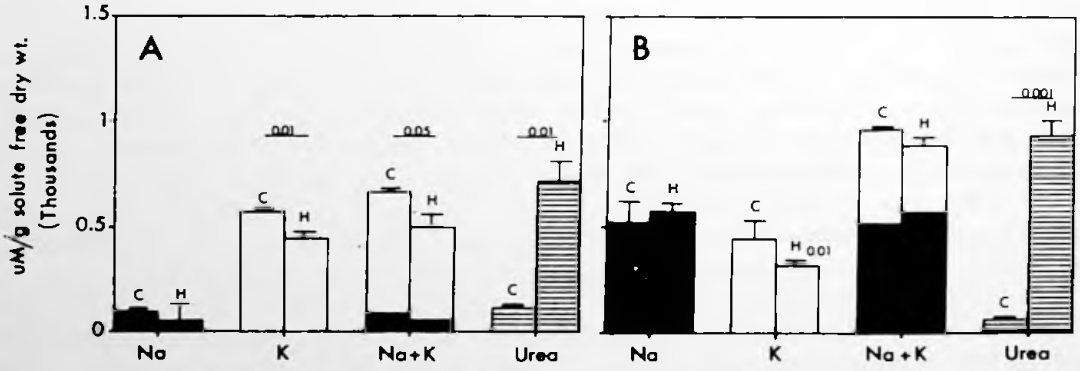

Figure 2. Steady state solute content (Mean + S.E.) in RPIC (A) or RPCT (B) acclimated to the Control Medium (C) or the High Na - High Urea Medium (H). Significance bars begin and end at values significantly different from each other ($P < 0.05 - 0.001$). No bar = significant loss ($P < 0.01$).

the 2 media were significantly different, In the RPCT, the loss of K content was significant. The net effect as measured in steady state was a constant intracellular K concentration (mM/l). Neither cell type changed significantly in Na content.

Urea content (μM/g sfdw) increased when both cell types were acclimated to the High Na - High Urea Medium (Figs. 2a, 2b). However, this change was not sufficient to account for the change in the total amount of osmotically active solutes in the RPIC or the RPCT. Based on calculation of an osmotic balance sheet, where we set the osmolality of the cells exactly equal to the osmolality of the medium in which we incubated them, unidentified osmolyte(s) (μOs/g sfdw) increased significantly in both cell types. In the RPIC the magnitude of the change in these osmolyte(s) was 514 ± 140 ($\underline{P}$ <0.02) whereas in the RPCT it was 1394 ± 205 ($\underline{P}$ <0.001).

Our steady state data indicate the utilization of urea during apparent regulatory volume increase. However, we do not know the short-term handling of urea in these cells or how the RPIC and RPCT differ considering the ability of the RPCT to hyporegulate urea concentration. Additionally, we do not know the nature of the unidentified osmolyte(s). It is necessary to identify these osmolyte(s), determine the time course in which these solute(s) can be mobilized, and determine the osmotic and solute concentrations under which these osmolytes are used. Our steady state data also indicate that RPIC and RPCT do not regulate water content via Na or K. However, from these data we can not tell whether the RPIC or the RPCT transiently use inorganic solutes during volume readjustment. This research was supported by NIH AM15972.