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Amino acids as well as polyols and methylamines accumulated in rat kidney during dehydration

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Summary. During antidiuresis cells in the renal inner medulla contain large amounts of sorbitol, myo-inositol, glycerophosphorylcholine and betaine to adjust the intracellular osmolality to the extracellular hyperosmolality. Although the accumulation of these four major organic osmolytes in the inner medulla of the dehydrated animal has been a consistent finding, the role of another class of organic osmolytes, amino acids, in osmoregulation in the kidney remains controversial. In the present study, renal responses of four major osmolytes and amino acids to dehydration were investigated using two HPLC systems. Taurine levels were significantly higher in the inner medulla of the dehydrated rats as compared with the control rats, and increased monotonically from the cortex to the inner medulla along the corticopapillary axis in the dehydrated rats. As for four major osmolytes, we confirm previously reported patterns in antidiuresis in greater detail. In conclusion, not only the four major osmolytes but taurine also plays a salient role in the osmoregulation in the kidney.

Keywords: Amino acids – Osmolytes – Dehydration – Rat – Taurine – Myo-inositol – Sorbitol – Betaine – Glycerophosphorylcholine

Introduction

Osmotically active organic solutes or "organic osmolytes" are known to be accumulated in the renal medullary cells to balance the extracellular hyperosmolality (Bagnasco et al., 1986). Cells apparently use these organic compounds to adjust their intracellular osmolality because, unlike "perturbing" solutes such as NaCl, KCl, and urea, the organic osmolytes inhibit enzymes and other cellular processes relatively little even at high concentrations (Yancey et al., 1982). The organic osmolytes fall mainly into three groups: polyols (such as sorbitol and myo-inositol), methylamines (such as glycerophosphorylcholine (GPC) and be-

taine), and amino acids (such as taurine, glycine and proline). Although, among osmolytes, the accumulation of polyols and methylamines in the renal inner medulla has been a consistent finding, the role of amino acids in osmoregulation in the kidney has remained controversial. Large quantities of amino acids are present in the renal medulla, but it has been questionable whether the levels of medullary amino acids are osmotically regulated.

As for other mammalian organs, considerable evidence suggests that taurine plays a salient role in osmoregulation in brain and heart (Thurston et al., 1980; 1981). Taurine is the most abundant free amino acid in animals and is well established as playing an important role in the maintenance of intracellular osmolal concentration in marine invertebrates, teleosts, and amphibians.

The renal epithelial cell line MDCK (Madin-Darby canine kidney) has been demonstrated to be a model for studying osmolyte accumulation in the renal inner medulla (Nakanishi et al., 1988; 1989). In these cells taurine uptake was stimulated by hypertonicity (500 mosmol/kg, by adding raffinose) (Uchida et al., 1990). These data suggested that taurine might function as an osmolyte to balance the extracellular hyperosmolality in the kidney cells.

To clarify the osmoregulation of amino acid accumulation in the kidney, we directly measured 26 different amino acids, as well as sorbitol, myo-inositol, GPC and betaine along the corticomedullary axis of rats that were given water ad libitum or were dehydrated by deprivation of water for 4 days.

Methods

Animals

Male Wistar rats, weighing approximately 280–380 g, were divided into two groups: Control and dehydrated. Control animals were given free access to water, whereas dehydrated rats were deprived of water for 96 hours. All animals were housed individually in metabolic cages and deprived of food during 2-day period prior to sacrifice. After metabolic cages were cleaned to minimize the contamination of feces and hairs in the urine collection flask, urine samples were collected during the 3 to 12-hour period prior to sacrifice. Rats were killed by decapitation under light ether anesthesia. Serum samples were collected at sacrifice.

Serum and urine sodium and urea concentration was measured by the autoanalyzer (Hitachi, TYPE-710 or TYPE 736, Tokyo, Japan). Serum and urine osmolality was measured with a freezing point osmometer (Kyoto-Daiichi-Kagaku, OM-6010, Kyoto, Japan).

Tissue extracts were prepared as reported previously (Gullans et al., 1988; Yancey et al., 1989). Kidneys were rapidly removed. They were sliced with scissors into three segments along the corticopapillary axis: cortex, outer medulla, and inner medulla. These sections were frozen in liquid nitrogen, lyophilized over 24 hours and weighed to obtain the dry weight.

These dried sections were homogenized with a glass homogenizer in 3 ml cold 7% perchloric acid (PCA). The homogenates were centrifuged for 20 min in the cold. The pellets were then dissolved in 1 N NaOH and analyzed for protein content using the Bio-Rad protein assay (Bio-Rad Labs, Richmond, Ca) with Bovine γ-globulin as a protein standard. The tissue water content was calculated by the difference between the wet and dry weights and divided by protein content to obtain water-to-protein ratio.

Measurements of tissue organic osmolytes

Betaine, GPC, sorbitol, myo-inositol and amino acid osmolytes were measured by high-performance liquid chromatography (HPLC) of PCA extracts of tissue (Wolff et al., 1989). The tissue supernatants from the centrifugation step were neutralized with 2.0 N KOH to

approximately pH 7, and passed through a Sep-Pak C18 cartridge (Waters, Milford, MA). In addition, extracts used to measure betaine and myo-inositol (but not GPC, sorbitol and amino acids) were also placed on a 1 ml AG1-X4 (hydroxyl form) column, and betaine and myo-inositol were eluted with 4 ml of water (unbuffered). This ion-exchange column retained unidentified peaks that otherwise appeared near betaine and myo-inositol on HPLC, but passed >99% of the betaine and myo-inositol (Nakanishi et al., 1990). The organic osmolytes were measured in the processed extracts by HPLC, with the use of a Sugar-Pak I column. Amino acids were measured with a High-Speed Amino Acid Analyzer (Hitachi L-8500, Tokyo, Japan) using the same samples diluted with the same volume of 0.04 N HCl.

Calculations and data presentation

The results shown are the mean ± SEM for seven to nine experiments carried out under the same conditions. Statistical significance was determined using the Bonferroni method (Wallenstein et al., 1980) for multiple comparisons or Student's *t* test for paired comparisons. Differences were considered statistically significant for $P < 0.05$.

Results

Urine and serum chemicals

Urine osmolality, urinary sodium, potassium and urea concentrations were significantly higher in dehydrated rats than in control rats (Table 1). The serum sodium and urea concentration, and osmolality were also significantly higher in dehydrated rats than in control.

Table 1. Urine and serum measurements in control and dehydrated rats

Measurement	Control	Dehydrated	P
Urine chemicals			
Na (mEq/L)	8 ± 3	65 ± 17	a
K (mEq/L)	44 ± 23	278 ± 26	a
Urea (mM)	452 ± 236	2163 ± 269	a
Osm. (mosmol/kg)	804 ± 343	3590 ± 399	a
Serum chemicals			
Na (mEq/L)	136 ± 2	160 ± 8	a
Urea (mM)	22 ± 2	41 ± 10	a
Osm. (mosmol/kg)	316 ± 6	336 ± 9	a

Urine samples from control or dehydrated rats were collected for the 3-hour or 12-hour period prior to sacrifice respectively. Serum samples were collected at sacrifice. *a* indicates significant difference between control and dehydrated rats.

Water content

Water content (water-to-protein ratio) in the inner medulla was significantly larger than that in the cortex or in the outer medulla, and rose monotonically from the cortex to the inner medulla in control and dehydrated rats (Table 2).

The water content in inner medulla from dehydrated rats was nearly the same as that from control.

Table 2. Intrarenal tissue distribution of water, polyol, and methylamine contents in control and dehydrated rats

	Control				Dehydrated			
	Cortex	Outer medulla	Inner medulla	P	Cortex	Outer medulla	Inner medulla	P
Water content	2.4 ± 0.1	3.6 ± 0.2	6.5 ± 0.5	a, b	2.1 ± 0.1	3.4 ± 0.1	6.8 ± 0.6	a, b
Polyols								
Inositol	6.7 ± 0.6	65.0 ± 8.3	103.9 ± 13.1	a, b	7.4 ± 0.4	92.1 ± 8.2	140.4 ± 13.6	a, b
Sorbitol	0.1 ± 0.1	0.6 ± 0.3	23.9 ± 3.9	a, b	0.4 ± 0.2	2.6 ± 0.9	43.2 ± 7.8	a, b c
Methylamines								
GPC	3.8 ± 0.7	17.0 ± 2.8	124.0 ± 24.8	a, b	6.1 ± 0.5	33.1 ± 4.3	267.2 ± 26.3	a, b c
Betaine	3.2 ± 0.7	35.8 ± 5.9	36.9 ± 4.3	a	3.8 ± 0.6	68.8 ± 5.5	59.5 ± 8.3	a c

Water content and osmolyte contents are expressed as L/kg protein and mmoles/kg protein, respectively. *a* or *b* in the table indicates a significant difference between the cortex and the inner medulla, or between the outer medulla and the inner medulla respectively. *c* indicates significant difference in the mean osmolyte content in the inner medulla between control and dehydrated rats.

Polyols and methylamines

Intrarenal tissue gradients of both polyols (sorbitol and myoinositol) and methylamines (betaine and GPC) were present, with the highest values toward the tip of the inner medulla (Table 2). Sorbitol was low throughout the cortex and outer medulla, but rose steeply in the inner medulla from both control and dehydrated rats. GPC rose a small extent from the cortex to the outer medulla, and steeply from the outer medulla to the inner medulla from both groups. Myo-inositol increased from the cortex to the outer medulla, and to the inner medulla monotonically from both groups. The betaine content rose from cortex to outer medulla by 10–20 times and then maintained an identical level or even diminished in the inner medulla.

Sorbitol, GPC, and betaine contents in inner medulla of dehydrated rats were significantly larger than those of control. In the outer medulla from dehydrated rats, on the other hand, GPC and betaine contents were significantly larger than those from control rats. Only GPC content in the cortex from control and dehydrated rats differed significantly, but not inositol, sorbitol, and betaine.

Amino acids

Among 26 amino acids quantitated by amino acid analyzer, the amino acids whose content in the kidney tissue was more than four mmoles/kg protein, i.e., taurine, glutamic acid, glutamine, glycine, aspartic acid, alanine, serine, proline, and phosphoethanolamine are discussed below (Table 3). Intrarenal tissue gradients for all amino acids except phosphoethanolamine were present with the highest values toward the tip of the inner medulla.

Table 3. Intrarenal tissue distribution of amino acids in control and dehydrated rats

	Control				Dehydrated			
	Cortex	Outer medulla	Inner medulla	P	Cortex	Outer medulla	Inner medulla	P
Taurine	20.7 ± 1.5	41.1 ± 3.7	38.6 ± 5.1	a	27.7 ± 3.2	47.2 ± 4.6	58.5 ± 5.6	a c
Glutamic Acid	10.6 ± 0.5	17.0 ± 1.1	19.6 ± 1.7	a	11.0 ± 0.8	17.1 ± 0.7	20.8 ± 1.6	a c
Glycine	7.7 ± 0.6	14.9 ± 1.1	13.6 ± 0.9	a	8.6 ± 0.7	17.1 ± 1.8	17.3 ± 1.7	a
Glutamine	2.7 ± 0.6	7.5 ± 2.3	21.7 ± 7.3	a	2.3 ± 0.5	13.1 ± 3.2	24.1 ± 5.9	a
Aspartic Acid	3.7 ± 0.6	8.1 ± 1.0	7.3 ± 0.6	a	3.9 ± 0.5	8.8 ± 1.0	8.8 ± 1.3	a
Alanine	2.4 ± 0.1	5.4 ± 0.5	7.6 ± 0.7	a, b	2.8 ± 0.4	5.5 ± 0.4	9.3 ± 1.4	a, b
Serine	2.1 ± 0.1	3.0 ± 0.2	3.8 ± 0.3	a	2.6 ± 0.4	3.7 ± 0.4	5.1 ± 0.6	a
Proline	1.1 ± 0.1	2.2 ± 0.3	3.2 ± 0.4	a	1.2 ± 0.2	2.3 ± 0.3	3.4 ± 0.4	a
PEA	6.3 ± 0.7	2.7 ± 1.4	0.9 ± 0.4	a	8.1 ± 0.6	4.3 ± 1.2	1.1 ± 0.4	a, b

PEA is a abbreviation for O-phosphoethanolamine. Amino acid contents are expressed as mmol/kg protein. *a* or *b* indicate a significant difference between the cortex and the inner medulla, or between the outer medulla and the inner medulla respectively. *c* indicates a significant difference in the mean osmolyte content in the inner medulla between control and dehydrated rats.

The taurine content doubled from cortex to outer medulla and then maintained an identical level in the inner medulla from the control rats. In the dehydrated animal, on the other hand, it increased from the cortex to inner medulla monotonically, but the taurine content in the inner medulla and the outer medullas did not differ significantly. The taurine content in the inner medulla from dehydrated rats was significantly higher than that from control. As for the content in the cortex, taurine and phosphoethanolamine contents in the cortex from dehydrated rats were higher than those from control, but not significantly ($p < 0.1$).

Discussion

Polyols and methylamines accumulation in the kidney

The patterns for polyols and methylamines in the rat kidney were similar to previously reported patterns in the rabbit kidney (Yancey et al. 1989). In dehydrated rats, the contents of sorbitol, GPC, and betaine were significantly larger than those from control.

Steady increases of the contents of inositol, sorbitol, and GPC from cortex to inner medulla were observed in dehydrated rats, but not betaine. Failure of the betaine content to increase from outer medulla to inner medulla might be related to high urea concentration in inner medulla, since high concentration of urea inhibit the betaine accumulation in MDCK cells (Nakanishi, 1990).

Amino acid accumulation in the kidney

Previous reports concerning the relationship between amino acid concentrations and osmolality in the kidney might include several determination errors. In these reports, amino acids concentrations were estimated from the amount of nin-

hydrin-positive substances. However not only amino acids but also urea and ammonia are ninhydrin-positive substances and the renal inner medulla contains large amounts of urea and ammonia. Gullans et al. (1988) found significant levels of ninhydrin-positive substances in renal inner medulla but did not detect the difference between control and dehydrated rats. Law et al. (1987) found the concentrations of ninhydrin-positive substances in renal papillary cells increased when medium osmolality was increased. If we recognize ninhydrin-positive substances as the sum of amino acids, we could not determine whether amino acids contents were osmoregulated or not.

Among amino acids quantitated by amino acid analyzer, the taurine content was the largest in the renal inner medulla. Taurine content in the inner medulla from the control rats was significantly larger than that from dehydrated rats. These data suggested that taurine content in the inner medulla was osmoregulated.

The sum of the inner medullary contents of amino acids other than taurine in Table 3 from control and from dehydrated rats (77.7 vs. 89.9 mmoles/kg protein) did not differ significantly. We could not determine whether amino acids other than taurine in the inner medulla was osmoregulated.

In the previous study, no content gradients of taurine along the corticopapillary axis were observed in the ruminant kidney, although taurine was a major amino acid component in the renal inner medulla (Robinson et al., 1966). The reason for the difference between the present and previous studies might be related to the species differences or to the method of determination. In their amino acid measurements the separation of amino acid was performed only by sulfonated cation exchange resin and not by high-performance liquid chromatography (HPLC) (Joseph et al., 1986).

In the present study, the taurine and phosphoethanolamine content of the cortex from the dehydrated rats were higher than those from control rats, but not significantly. Wolff et al. (1989) also obtained the similar result on rabbits study using ^{31}P-NMR. Phosphoethanolamine was mainly accumulated in the cortex from antidiuretic rabbits and the phosphoethanolamine content decreased when infused with isotonic solution. We did not know why phosphoethanolamine was mainly accumulated in the cortex, and not in the inner medulla, although the metabolism of ethanolamine, phosphoethanolamine, and phosphatidylethanolamine might be related to it.

Nonperfused proximal S2 segments from rabbit kidney cortex have been shown to keep cell volume constant if medium osmolality is elevated. Cell volume regulation was not fully accounted for by the addition of K^+, Na^+, Cl^- to the intracellular fluid. Grantham et al. (1989) suggested organic osmolytes were possible candidates to compensate the osmolality gap between intra- and extracellular osmolality. Because the contents of inositol, sorbitol, and betaine in the cortex from control and dehydrates rats did not differ, GPC, taurine and phosphoethanolamine might be strong candidate for adjusting the intracellular osmolality to the extracellular hyperosmolality in the cortex.

In the brain and heart of the mammalians, the role of amino acids in the osmoregulation has been established (Thurston et al., 1980; 1981), that is, the intracellular accumulation of amino acids might balance the extracellular

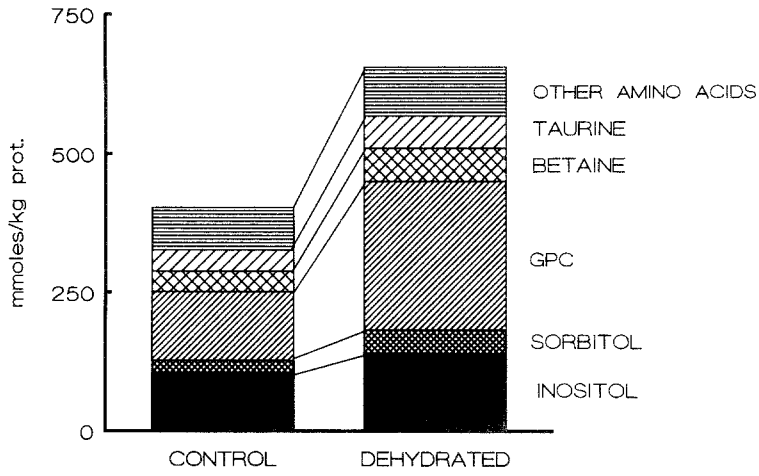

Fig. 1. Total and individual osmolyte contents of renal inner medulla from control and dehydrated rats. The content of other amino acids consist of glutamic acid, glycine, glutamine, aspartic acid, alanine, serine, and proline

hyperosmolality. The ratio of total amino acids to total organic osmolytes in the inner medulla was 28.6% from the control and 22.4% from the dehydrated rats (Fig. 1). The renal inner medulla should be exposed to the highest tolerable osmolality in the body when producing a concentrated urine. Although urine osmolality, urea and electrolytes concentration from dehydrated rats were 4–8 times greater than those from control rats, the difference of amino acid content in the renal inner medulla between control and dehydrated rats was not enough to compensate for that of urine osmolality. From the evolutional point of view, we supposed that renal medullary cells have gained the ability to accumulate several kinds of organic osmolytes, that is, polyols and methylamines, in addition to amino acids.

In conclusion, the rat renal inner medulla contains not only high concentrations of polyols and methylamines but also substantial amounts of amino acids as a concomitant of the renal concentrating mechanism. The taurine content especially in the inner medulla is highly regulated by hyperosmolality consistent with previous observation in brain and heart. Kidney cells can use amino acids for the purpose of adjusting the intracellular osmolality in the same manner as in bacteria, plants, and other organs (Yancey et al., 1982).

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